Monitoring the conservation status of natterjack toad (*Bufo calamita*) in Ireland, 2004 - 2006

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Monitoring the conservation status
of natterjack toad (*Bufo calamita*) in Ireland, 2004 – 2006

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Cover photo: Natterjack toads (*Bufo calamita*) in amplexus © Aurélie Aubry

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Executive summary

- Natterjack toad breeding activity for the years 2004, 2005 and 2006 is recorded for eleven of the twelve known breeding sites in South West Ireland. A summary of this breeding activity is given for all sites visited for each of the three years.

- Egg and tadpole survival rates are calculated for each of the breeding ponds and each toad population (on a site by site basis), based on the census data recorded in the three years.

- Geographical fluctuations in population size, breeding activity intensity and demography are analysed and compared between the three years for which surveys have been completed.

- Population growth rates are calculated (based on a stage classified model) and sensitivity to toad life cycle stages are assessed.

- Effects of environmental factors on the breeding activity (density of egg strings) and success (egg and egg to toadlet survival rates) of natterjack toads across the three years are investigated and environmental indicators identified.

- A practical monitoring protocol for the species in Ireland is given

- Management recommendations for natterjack toad conservation in Ireland are given for each breeding site.

CONTENTS

1. INTRODUCTION

The natterjack toad (*Bufo calamita*) is a protected species listed on Annex IV of the Habitats & Species Directive (92/43/EEC), this requires member states to implement a system of strict protection to maintain a favourable conservation status for the species. Although common in parts of its European range, *B. calamita* is restricted to twelve coastal areas of County Kerry, where they were first recorded by Mackay in Callinafersy in 1805 (Whilde 1993), and one site in Wexford where they were translocated by NPWS in the early 1990s (F. Marnell pers. comm.).

This report represents the outcome of a three-year study (2004-2006) of natterjack populations at all but one of the known breeding sites in County Kerry. Due to sensitivities over access, the Inch Peninsula breeding site was not included in the present survey. This study, commissioned by the National Parks and Wildlife Service (NPWS), arises from a recommendation by Beebee (2002) that a dedicated monitoring programme be instigated for the toads in County Kerry. Our study aims to assess the conservation status of Irish toad populations, and provides a baseline against which to compare future trends in natterjack populations in Ireland.

Regular surveys of all known breeding sites were undertaken from 2nd April to 31st July each year (2004, 2005 and 2006). The ephemeral nature of the natterjack breeding ponds means that the number of breeding ponds varies between years and among sites. In addition, features of ponds such as pond size, inter-pond distance and degree of pond permanency vary depending to rainfall in different years.

Each annual survey documented the biotic and abiotic factors that affect the distribution, local population sizes and breeding success of the toads. Specifically, the main objectives were to:

1. Identify natural fluctuations in toad population dynamics
2. Identify the most suitable part of the life cycle for use as an indicator of conservation status
3. Identify the most suitable environmental indicators that affect the conservation status of the species
4. Provide a practical monitoring protocol for the species in Ireland.

2. METHODS

2.1 Breeding ponds

The breeding sites surveyed in 2004, 2005 and 2006 were:

On the North coast of the Dingle Peninsula: Fermoyle (3 ponds), Stradbally golf course (6 ponds + drains, and 1 additional pond in 2006), Lough Gill (2 areas), The Maharees (maximum of 10 ponds depending on rainfall), Tullaree (3 ponds).

On the South coast of the Dingle Peninsula: Roscullen Island (3 areas).

On the North coast of the Iveragh Peninsula: Lough Yganavan (5 areas), Lough Nambrackdarrig (North East shore), Dooks golf course (6 ponds), Glenbeigh quarry (approx. 10 pools), Glenbeigh field and Glenbeigh salt marsh.

On the South coast of the Iveragh Peninsula: Caherdaniel (2 ponds).

These sites are all shown in Figure 2.1.

In addition to the main sites a number of new areas were monitored: at Dooks, a small area of silt laden water, connected to Pond 6 (in the field North East from the golf course), was used for breeding in 2005 and 2006; egg strings recorded in this area were included in the total breeding activity of Pond 6. At Tullaree, a small ephemeral pond approx. 100m from the main pools was also used for breeding in 2004 and 2005.

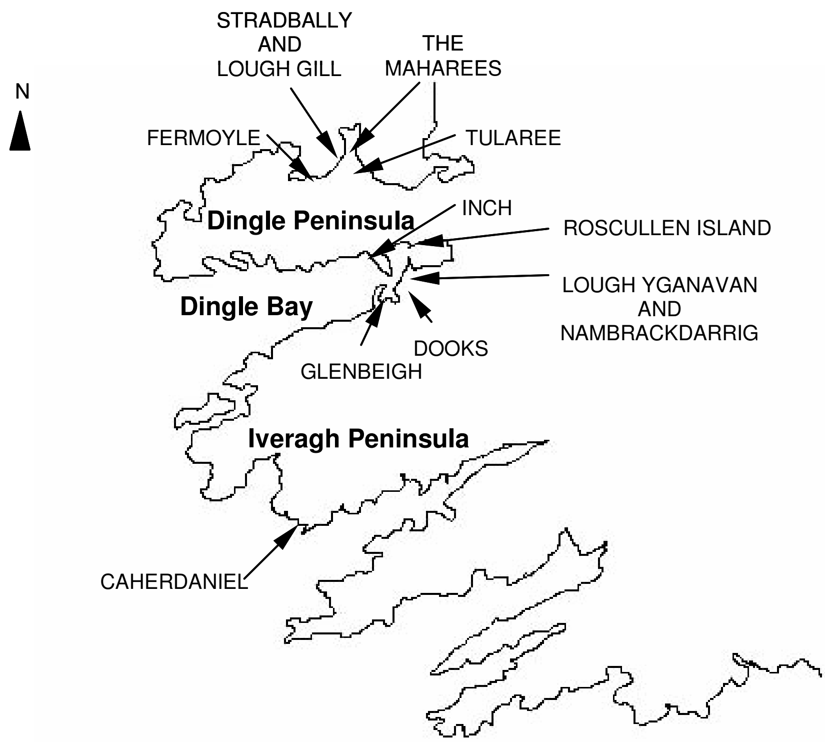


Figure 2.1. Distribution of Natterjack toad breeding sites in County Kerry, Ireland

2.2 Biotic parameters related to population dynamics of *B. calamita* populations

All breeding ponds at each of the eleven study sites were surveyed regularly (at 8-11 day intervals) from 2nd April to 31st July 2004, 2005 and 2006. Some tadpoles were still present in a few breeding ponds at Stradbally after the 31st July. Additional visits showed that by the end of August there were no tadpoles left in the ponds (except in 2004 at Stradbally where low 10s of tadpoles had still not metamorphosed). Site visits were carried out every 8-11 days in order to obtain accurate overall counts of egg strings, as egg strings normally hatch after 7 to 10 days (Beebee 1983). Later in the breeding season, when toadlets started to emerge and disperse, the frequency of site visits increased, and took place, at most, 7 days apart. At each visit, the following parameters were recorded (when appropriate):

2.2.1 Number of egg strings

The shallow parts of each pond were carefully surveyed for egg strings. Surveys consisted of walking slowly along the perimeter of the pond and visually scanning the margins of the pond. On occasion egg strings were also laid further away from the pond edges. When such egg strings were detected and water depth permitted, transects spaced at four metre intervals were followed between two opposite edges of the pond, taking care to avoid disturbing the pond substratum and vegetation.

The number of egg strings was recorded for each pond. Recently laid egg strings consisted of double lines of eggs, in contrast older strings containing well-developed eggs had a single line of eggs. The position of individual strings was recorded on a sketch map of each pond. This enabled the surveyor to identify any strings that were still present during subsequent visits and thus avoided double counting. The number of egg strings presented in this report is a cumulative count of the egg strings recorded throughout the breeding season at each pond.

The depths of some ponds or their abundant vegetation prohibited a thorough survey of the entire pond area, and some egg strings may have been missed. Local pond features may have contributed to a miscount of egg strings, e.g. if egg strings were laid away from the edges of ponds or within areas of high vegetation cover. This was the case in ponds containing emergent macrophytes such as Chara or *Potamogeton* spp., where toads were able to access the entire surface area of the pond for breeding (in particular at Roscullen, Dooks field and Tullaree).

2.2.2 Number of tadpoles

Tadpoles were usually found in the shallower parts of the ponds. We assessed their numbers by counting the visible tadpoles, as recommended by Denton *et al.* . (1995) and Raw & Pilkington (1988). In many instances, tadpoles were aggregated in large numbers and at variable densities within the same pond. Therefore their numbers were assessed by multiplying the estimated number of tadpoles per m^2 by the area where they were present at this density. The area was estimated visually with the use of a quadrat. Unlike common toad tadpoles, natterjack tadpoles are quite visible (i.e. not hidden in silt, under stones or in deep waters) during most of their development (Beebee 1985). However, personal field observations and other studies (e.g. Beebee 1985) show that shortly before metamorphosis, large tadpoles tend to remain hidden, resulting in likely underestimations of their abundance at that stage. It would have been too time consuming, and probably disturbing, to estimate the number of tadpoles using a net. In some ponds, densities varied from 10s to 100s of tadpoles per m^2.

In some ponds where the water was not clear and/or the vegetation very dense and abundant, egg strings and tadpoles were not always easy to detect (e.g. at Tullaree, Roscullen Island, some parts of Lough Yganavan, Lough Nambrackdarrig, Ponds 4-5 at Dooks and in Pond 2 at Glenbeigh). To limit the underestimation of tadpoles at these ponds, a small, flat, white paddle was used to help spot egg strings and tadpoles in the water.

The common toad *Bufo bufo* is not present in Ireland, and consequently there was no possibility for confusion when identifying tadpoles. Tadpoles of the common frog *Rana temporaria* were present at many of the breeding ponds. However, the frog-spawning period ended well before the natterjack toads started spawning. Large frog tadpoles have been observed at several ponds in this study and could be easily distinguished from toad tadpoles by their olive-brown colour, speckled appearance and pointed tail (when undamaged) (Gent & Gibson 1998, Herpetological Conservation Trust (HCT) 2003). On the contrary, natterjack toad tadpoles have a uniform black appearance, a rounded tail and are relatively small; they grow to a length of about 25 mm. From the beginning of the survey period each year (2nd April), no frogspawn was observed in any of the breeding ponds.

2.2.3 Number of toadlets

Knowledge of toadlet production is important and gives an immediate indication of the site viability: failure to produce toadlets for two or three years probably indicates that a serious problem has arisen (HCT 2003). However, it is not possible to record the number of toadlets with a high degree of accuracy. Although regular visits to breeding ponds enable the recording of large tadpoles (with long hind legs, a sign of imminent metamorphosis) toadlets can rapidly disperse from the pond when weather conditions permit (HCT 2003) and many can thus be easily missed.

Previous scientific studies (e.g. Denton 1991, Gent & Gibson 1998) estimated toadlet production using pitfall traps. However, this method requires frequent visits and is not feasible when monitoring many sites. HCT (2003) recommend using estimates at least to an order of magnitude (0, 10s, 100s, 1000s), the approach has been widely applied in previous studies (e.g. Beebee & Buckley 2001).

In the 2004 to 2006 surveys, random quadrats (50cm by 50cm) were used to estimate the number of toadlets in the vicinity of each pond: the surface area concerned was measured in length and width (using a tape measure) and multiplied by density estimates to obtain numbers per pond. Random number tables were used (to avoid sampling errors) to position quadrats within this area and counts of toadlets were made within these quadrats. In each case, the distance surveyed from the edge of the pond did not exceed 20m. We assumed that at distances greater than 20m only old toadlets (therefore already counted in previous surveys) would be observed. Toadlets were counted in six quadrats and their mean density per m^2 estimated from these counts. The overall number of toadlets was then estimated by multiplying the toadlet density by the surface area of adjacent habitat under survey. When toadlets were gathered in distinct groups (typically when they had just emerged), the size of all observed groups was recorded and used as an index of absolute abundance.

Toadlets can be easily distinguished from froglets. They are usually black at emergence but soon develop typical natterjack colouration with the characteristic yellow line along the back of the toad. The gait of the species also makes them easy to recognise, froglets typically jump if disturbed, whereas toadlets only crawl to escape.

2.2.4 Number of adults

Counts of egg strings give a reliable indication of the number of adult breeding females in the population (Banks & Beebee 1988, Gent & Gibson 1998). If the sex ratio of a population is known, an estimate of the number of mature males can also be obtained.

The number of egg strings recorded in any one year may not equal the number of mature adult females in the population, for example, previous studies have shown that not all females spawn every year (e.g. Denton 1991, Denton & Beebee 1993, Stephan *et al.* 2001). An estimate of the proportion of adult breeding females is not available for the Co. Kerry populations. Aubry & Emmerson (2004) reviewed the proportion of adult breeding females reported in similar studies across Europe. This review showed that a substantial proportion of adult females do breed each year, on average 65% of the adult female population. Double clutching by natterjack toads can occur, and has been reported in UK and Swedish populations (Denton 1991; Silverin & Andren, 1992; Denton and Beebee, 1996). The incidence of double clutching is, however, considered to be relatively low within populations (Denton 1991; Silverin & Andren, 1992; Denton and Beebee, 1996). Although we are unable to estimate the incidence of double clutching at Irish breeding sites, we discuss its likelihood based on the 2004 to 2006 data (see section 3.2). We defined adult female population size as $F = S/0.65$, where F is the adult female population size and S the number of observed strings in the breeding season. In the absence of more detailed information, we assumed a 1:1 sex ratio in the Co. Kerry populations and the estimated number of adult females was thus doubled to give an estimate of the total adult population size. It should be noted that often, male mortality rates are higher than female mortality rates), and the sex ratio can be skewed towards females (Denton 1991). If this were the case for Irish populations, then population sizes reported here represent an overestimate.

2.2.5 Number of eggs per string

In order to assess the fertility of females, the number of eggs in a sub-sample of fresh strings was counted at most breeding sites. Whenever possible, these strings were gently taken out of the water and placed in a flat white tray filled with pond water, photographed (digitally) and immediately replaced in the original location. Egg counts were subsequently made from digital images using the software ImageJ (version 1.34s). In many instances the eggs overlap each other, the total number of eggs was therefore estimated by dividing the total surface area represented by the eggs by the average size of an egg (specific to each photograph). We assessed the accuracy of this technique by counting the number of eggs manually from the printed photographs of a subsample of egg strings selected at random, representing 8.5% of all the egg strings analysed.

The average number of eggs per string was then obtained for each pond sampled at different sites, as well as a global average for the Co. Kerry natterjack populations. The average number of eggs per string (specific to each site) was then used to obtain a more accurate estimate of the egg to toadlet survival rate on a per site basis (see section 3.4).

Although only fresh strings (i.e. those still in double lines) were analysed, some egg mortality may occur through predation, which means that the number of eggs counted per string is a conservative estimate of female fertility. In some instances, images were taken of the egg strings *in situ* (without disturbing the string). This method proved to be inaccurate and the images difficult to analyse, with many eggs not showing clearly in the images (due to variations in water depth, vegetation cover, brightness and reflection). For comparative purposes the fertility rates of natterjacks elsewhere in Europe were also reviewed from the literature.

2.3 Environmental parameters

Several environmental parameters were recorded at each site visit. The pH, conductivity and water temperature were measured using a pH/Conductivity meter (Model WTW – Multiline 340i) that was accurate to ± 0.01 pH units and 1 $\mu S. cm^{-1}$ for conductivity values. The reference temperature for the conductivity probe was 25°C. When the conductivity was high (> 800 $\mu S. cm^{-1}$), salinity was also measured, using the pH/Conductivity meter. The pH electrode was calibrated each morning preceding each survey. Environmental measures were taken close to the pond edge (usually within the first metre) where eggs or tadpoles were present, avoiding areas where plants and algae were abundant. Local variations in water chemistry may occur due to subtle changes in vegetation type and abundance and changes in water depth. Consequently, three measures of pH and conductivity were taken at each location (within 3 m^2) and averaged. For pH (represented on a $\log_{10}$ scale), measurements were transformed to hydrogen ion concentrations, then averaged and finally back transformed to the logarithmic scale.

Water depth (from the bottom of the pond to the water surface) was measured to the nearest 0.5 cm using a metre stick at each location where pH and conductivity were taken, the time was noted and the air temperature recorded using a hand held thermometer.

At the first visit to each pond, the different land use and management practices surrounding the pond were noted. Any changes that occurred over the course of the breeding season were recorded during subsequent visits (see NPWS Access database). The intensity of the activities (mainly grazing and cattle poaching) was also noted.

The surface area of all ponds varied throughout the breeding season, and information on significant decreases in pond area was assessed visually and noted.

In addition to the continuous variables mentioned above, we also considered three categorical variables for each pond. "Habitat" represents the predominant habitat type surrounding each pond. The different types are (1) Early sand dune, (2) Scrub (includes sand dunes in late stages), (3) Bog and Heathland, (4) Improved grassland and (5) Marsh. "Activity" refers to the predominant form of land use occurring in the area adjacent to the ponds, and the different categories are (1) Farming activity, (2) Golf course and (3) Discontinuous land use (i.e. low degree of human intervention). Lastly, "pond permanency" refers to the hydroperiod of each pond, classified as (1) permanent, (2) semi-permanent (reduced by at least half its size of April before the end of the breeding season (31st July)) and (3) ephemeral (dried out before the end of the breeding season). The values obtained for these three categorical variables are presented in Appendix 1.

2.4 Determination of survival rates

2.4.1 Techniques for calculating survival rates (method selection)

Commonly field herpetologists assess the breeding activity and success of amphibian populations by quantifying trends in spawning activity and in the production of metamorphs, over several years of survey (Buckley & Beebee 2004, Kupfer & Kneitz 2000). Regular surveys are needed to gain an accurate estimation of the total number of eggs laid in the pond and of the total number of metamorphs produced within a given year. Regular surveys are also needed to calculate the survival rate from eggs to metamorphs (especially when the focal species has an extended breeding season). Natterjack toads have a particularly extended breeding season (they can lay eggs from April to August), which results in an important overlap of successive generations of tadpoles. It is thus not possible to accurately assess the total number of toadlets emerging from the pond. Each successive count includes some newly metamorphosed individuals and also a proportion of toadlets that may not have yet dispersed away from the breeding pond (since the last count). The estimation of the egg to toadlet survival is therefore not straightforward.

A good estimation of the number of metamorphs emerging at a given pond is possible when (1) newly metamorphosed toadlets are easily recognisable from older toadlets (due to their different coloration and sizes) or (2) where most of the tadpoles metamorphose during the same week (as occurred in 2006 at the Maharees Pond 23). Our observations show that in ponds with high densities of tadpoles (in particular at Stradbally and Tullaree) it is relatively easy to distinguish newly metamorphosed toadlets from older toadlets. The newly metamorphosed toadlets are often very small (<1cm SVL) and still completely black. In contrast, at sites such as Roscullen Island and Dooks, where toadlet emergence occurred over several weeks, it was not possible to distinguish new toadlets from older toadlets (emerging toadlets were relatively large (>1.2cm SVL) and also possessed adult coloration at metamorphosis and emergence).

For each year (2004 to 2006) the census data for each breeding pond, and at each site visit, represent counts and estimates of the numbers of individuals in four different developmental stages (egg, tadpole, toadlet, and adult). This type of data is often referred to as stage-frequency data (e.g. Manly 1989). The production of eggs by natterjacks does not occur in a single pulse, and consequently natterjack toad life history data is most accurately quantified as "multi-cohort stage-frequency data", according to Manly (1989).

The stage frequency data collected in the present study do not represent the natterjack toad's full life cycle. The adult toad population size is estimated using the number of egg strings counted per pond as a proxy for female population size. No empirical estimate of adult survival rate is available for Irish populations. Additionally, the numbers of subadults (including the ages 1-4 years) in the population remain unknown, confounding the estimation of life history parameters for subadults and adults at Irish breeding sites. A number of methods do exist for estimating the demographics of whole populations (e.g. Wood 1994, Gross *et al.* 2005, Nelson *et al.* 2004, Tuljapurkar & Horvitz 2006). Unfortunately, these cannot be applied in the present study without a detailed knowledge of the age structure of the Irish toad populations.

The data collected in successive years (2004-2006) from 1st April to 31st July represent counts of a cohort of individuals all hatching within the same period (here a few weeks) and developing through a series of stages (tadpoles and toadlets) without reproducing. Our goal is to determine the survival rate (from eggs to toadlets) by counting the numbers of tadpoles and toadlets during successive surveys. Stage frequency methods are employed for the analysis of such data (Caswell 2001).

A number of methods have been proposed for analysing multi-cohort-frequency data. These methods vary in their simplifying assumptions and mathematical complexity and each has associated pros and cons. None of the available methods accurately describe the data collected in the present study and so it is necessary to make some simplifying assumptions. Manly (1989) compared the outcomes of more than 20 different methods using simulated stage structured population data. A number of these approaches are presented in Table 2.1 along with a critical evaluation of each. Given the constraints of the data collected in the present study (i.e. multi-cohort-frequency data), we find that the Kiritani-Nakasuji-Manly method (KNM method) is the most parsimonious (in terms of limiting assumptions) whilst retaining an appropriate level of mathematical complexity. Manly (1989) concluded that when the daily survival rate is constant with time and from stage to stage, the Kiritani-Nakasuji-Manly method (KNM method) provides good estimates of survival rates.

In the present natterjack case, it is unlikely that survival rate per unit time is the same for all stages (eggs and tadpoles) at all times. For example, it is likely that as tadpoles grow larger, the probability that they will survive increases, especially as they become less vulnerable to predation (Chase 2003, Denton & Beebee 1997). However, the methods that allow survival rate to vary with time and stage of development require more information than just the stage-frequencies, e.g. the exact distribution of entry in stage 1 (i.e. single- vs. multi-pulse breeding) or the mean duration of each stage (Manly 1989, Bellows & Birley 1981, Manly 1997). Given the important overlap of adult generations in *B. calamita* populations, the lack of a clearly defined breeding pulse, and the fact that the time to metamorphosis (and thus the duration of the tadpole stage) varies strongly with the weather conditions (e.g. Alvarez & Nicieza 2002, Beebee 2002), our data do not allow us to accurately estimate these other population parameters. We have thus used the KNM method in this report to estimate the egg and tadpole survival rates of natterjack toads. The KNM method provides a conservative estimate of survival, and although the method assumes a constant daily survival rate in each pond, its use is valid when comparing survival rates across different ponds and relating these survival rates to the local environmental conditions.

Table 2.1.: Advantages and limitations of stage frequency methods used for estimating stage survival rates. Adapted from Manly (1989).

Name	Type	References	Advantages	Limitations
Richards and Waloff (1954) method	Regression model.	Richards and Waloff (1954), Manly (1989), Southwood and Henderson (2000)	• Low mathematical complexity • Does not require any information in addition to the stage frequency data	• Assumes that the survival rate per unit time is the same for all stages • Assumes a single pulse of recruitment
Kiritani-Nakasuji-Manly (KNM) method	Graphical method	Kiritani and Nakasuiji (1967), Manly (1976), Manly (1989), Yamamura (1998), Southwood and Henderson (2000)	• Low mathematical complexity • Does not require any information in addition to the stage frequency data	• Assumes that the survival rate per unit time is the same for all stages
Kempton (1979) Method	Maximum likelihood model.	Kempton (1979) in Manly (1989), Manly and Seyb (1989), Southwood and Henderson (2000)	• Flexible method, allowing some parameters to vary with time and among stages.	• Requires knowledge of the distribution of the survival rates (e.g. lognormal), which is the same for all stages • Requires knowledge of the distribution for the time of entry to stage 1 • Requires knowledge of the distribution for the duration of each stage • Mathematical complexity, requiring access to a computer program (e.g. MAXLIK)
Bellows and Birley (1981) method	Regression model	Bellows and Birley (1981), Manly (1989), Southwood and Henderson (2000)	• Allows different survival rates for each stage.	• Requires knowledge of the distribution of times of entry to stage 1. • Requires knowledge of the distribution for the duration of each stage
Manly (1987) method	Regression model	Manly (1987), Manly (1989), Manly and Seyb (1989), Wood and Nisbet (1991), Wood (1994), Manly (1997).	• Allows the survival rate per unit time to vary from stage to stage	• Does not provide relevant estimates based on simulation data (Wood and Nisbet (1991), Manly (1997)) • The error in the estimation of one survival parameter will be amplified when estimating other parameters (=propagation of error) • Mathematical complexity, requiring access to a computer program (e.g. MAXLIK)
Wood (1994)	Surface model using	Wood and Nisbet (1991),	• Allows survival rates to vary with	• Requires stage durations to be known.

method	the McKendrick -von Foerster equation and spline functions	Wood (1994)	time and among stages. • Limits the propagation of the errors involved in estimating the survival rate in one stage to subsequent ones.	• Mathematical complexity requiring heavy programming
Ratio of the number of toadlets divided by the number of eggs	Simple ratio, no model required		• Low mathematical complexity • Does not require any assumptions on the population parameters	• Requires the differentiation between old and newly emerged toadlets.

2.4.2 The KNM method

In the present study, three stages are defined: (1) eggs, (2) tadpoles, and (3) toadlets. To assess survival, the KNM method requires an estimate of the total number of eggs laid. The number of eggs per string can vary from site-to-site and country-to-country. Therefore to obtain an estimate of the total number of eggs laid at a given site it is necessary to multiply the average number of eggs present in a string, at a site, by the number of egg strings counted at that site. Aubry & Emmerson (2004) estimated that for Irish populations there were approximately 2,000 eggs per string (in line with an estimate obtained for natterjacks in the literature). In this report, a more accurate estimate of the fertility of female natterjack toads at Irish sites was obtained. This estimate is based on the number of eggs per string counted for a substantial number of egg strings (222) laid in 2006 (at each of the different breeding ponds; see section 3.3.1.). The results indicate that across all sites the mean number of eggs per string was 2,195 eggs, which is consistent with the assumption of 2,000 eggs made in 2004. However, there were significant local site differences (see section 3.3.1.).

Let j refer to one of the three stages eggs, tadpoles or toadlets (j = 1, 2 or 3). Let:

A_j = estimated area under the stage-frequency curve for stage j
h_i = time interval between survey i and previous survey (i-1)
n = the total number of surveys, with stage-frequencies being zero at the first survey (sampling occasion prior to the first occasion when individuals in that stage were recorded) and the last survey (end of breeding season, all larval stages have either died or metamorphosed).

A_j can be estimated using the trapezoidal rule:

$$A_j = \frac{1}{2} \times \sum_{1}^{n-1} \left(h_i + h_{i+1}\right) \times f_{ji} \qquad \textbf{(1)}$$

where f_{ji} is the number of individuals in stage j at the i^{th} occasion.

When assuming a constant daily survival rate, it appears that the area under the stage frequency curve (i.e. A_j, established from field census data and calculated as above) conforms to the area under the stage frequency curve that we would expect if all eggs were laid at the same time. Consequently, for each stage, A_j, can also be expressed as a function of the daily survival rate. The survival rate of the jth stage (for j = 1 (eggs) or 2 (tadpoles)) can then be defined by a simple ratio (see Manly [1989] for a derivation of the ratio). The numerator of the ratio is the sum of the areas for stages greater than j. The denominator is the sum of the areas for stages greater or equal to j, e.g. the egg survival rate is calculated as follows:

$$\phi_1 = \frac{A_2 + A_3}{A_1 + A_2 + A_3} \qquad \textbf{(2)}$$

In general terms, the survival rate of the jth stage is obtained as follows:

$$\phi_j = \frac{\sum_{i=j+1}^{q} A_i}{\sum_{i=j}^{q} A_i} = 1 - \frac{A_j}{\sum_{i=j}^{q} A_i} \quad \textbf{(3)}$$

where q is the total number of stages (in the present study $q = 3$).

We refer to Manly (1989) and Yamamura (1998) for further details on the KNM method.

In the present report the survival rates of eggs, tadpoles, and the overall survival from eggs to toadlets (i.e. egg survival × tadpole survival) are presented using estimates of site-specific fertility rates (when available), that is, the number of eggs per string. When a site-specific fertility rate is unavailable, the global average across sites has been used (number of eggs per string = 2,195).

2.4.3 *How to assess the suitability of the KNM method*

A comparison between the survival rates obtained using the KNM method and the simple ratio toadlets:eggs was carried our for ponds with large populations (see section 3.4.1.1. below) and indicates a strong correspondence between the two methods. The estimation of emerged metamorphs can lead to double counting of individuals. Consequently, the use of the ratio toadlets:eggs is not appropriate at all sites as a measure of survival and so the KNM method was chosen and applied to toad populations at all ponds.

Our surveys provide a *best estimate* of the number of eggs per string produced on a site-by-site basis and hence site specific fertility rates. To investigate the sensitivity of the KNM method to the value chosen for the fertility, we tested (paired *t*-tests) whether the survival rates obtained for each pond differed significantly using two fertility rate scenarios. First, we used fertility values estimated from field samples; second we used a fixed fertility rate value of 2,000 eggs per string (corresponding to the Aubry and Emmerson (2004) estimate). The fertility rates for each pond correspond to the average number of eggs counted in a sample of egg strings ($n \geq 5$). When it was not possible to process more than 5 egg strings per pond, the pond specific fertility rate was set equal to the global Irish average (i.e. 2,195 eggs).

Lastly, because for all ponds we have 1) an accurate estimate of the total number of egg strings produced (n_{strings}), and 2) the mean number of eggs per string, it is possible to obtain an estimate of the duration of each stage as detailed in Manly (1976).

The number of individuals entering the first stage (egg) can be estimated as follows:

$$n_1 = n_{strings} \times fertility \quad \textbf{(4)}$$

where *fertility* is set as the estimated value specific to each site.

The number of individuals entering the tadpole stage can then be obtained:

$$n_2 = n_1 \times \phi_1 \qquad \textbf{(5)}$$

where ϕ_1 is the egg survival rate as estimated by the KNM method.

If we consider that

$$n_2 = -\log\phi \times (A_1 + A_2) \qquad \textbf{(6)}$$

(see Richards *et al.* 1960 in Manly 1976) where ϕ is the daily survival rate and A_j, the area under the frequency curve as defined above, then we can obtain an estimate of $\log\phi$ from equations (1) and (2).

The duration of the egg and tadpole stages can then be estimated as follow:

$$d_1 = \frac{\log\phi_1}{\log\phi} \quad \text{and} \quad d_2 = \frac{\log\phi_2}{\log\phi}$$

It must be noted however, that the primary aim of the approach used in the present study (based on KNM model) is not to estimate the duration of each stage. Our aim here is to use the estimated durations for each stage as a way to assess whether the model provides realistic stage durations relative to observed stage durations.

2.5 Stage based population model and sensitivity analysis

2.5.1 Life cycle graph and associated matrix model

In order to study the demographic behaviour of natterjack toad populations, we described their life cycle using a stage-classified model. We make some simplifying assumptions: the model only projects the female breeding population (assuming a 1:1 sex ratio) using an annual time-step, so that

$$N_{t+1} = \boldsymbol{A}N_t \qquad \textbf{(7)}$$

where N_t is a vector containing the number of individuals in each stage at time *t*, and N_{t+1} is the number of individuals in each stage at the next time step. ***A*** is the population projection matrix containing the fertility rates and transition probabilities from one stage to the next. These stages are described in Figure 2.2 below. We assume that females breed for the first time at the age of four years (i.e. after having gone through their fourth winter) and that only 65% of the mature females breed each year (see 2.2.4.).

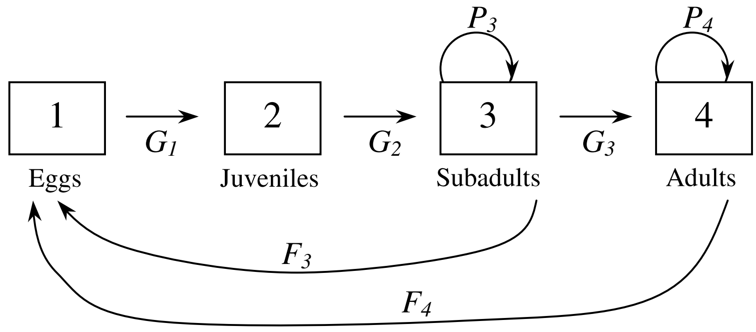


Figure 2.2. Life cycle graph for a natterjack toad population. Nodes represent stages: 1 = Eggs, 2 = Juveniles (survived through the first winter), 3 = Subadults (survived through the second and third winters), and 4 = Adults. *Fi* is the fertility of stage *i*, *Gi* is the probability of surviving and growing from stage *i* to stage *i*+1, and *Pi* is the probability of surviving and staying in stage *i*.

The projection matrix ***A*** corresponding to the graph in Figure 2.2 is

$$\boldsymbol{A} = \begin{bmatrix} 0 & 0 & F_3 & F_4 \\ G_1 & 0 & 0 & 0 \\ 0 & G_2 & P_3 & 0 \\ 0 & 0 & G_3 & P_4 \end{bmatrix}$$

In ***A***, the term F_i denotes the fertility of stage *i*, G_i is the probability of surviving and growing from stage *i* to stage *i*+1, and P_i is the probability of surviving and staying in stage *i*. This matrix, ***A***, whose elements are the population demographic rates, is often referred to as the transition matrix or Leslie matrix, and has been widely used to model the stage structure of amphibians (e.g. Griffiths & Williams 2000, Stephan *et al.* . 2001), insects (e.g. Schtickzelle & Baguette 2004) and reptiles (e.g. Crouse *et al.* . 1987).

For each site, G_1 was obtained from the site-specific survival rates of eggs and tadpoles. These site-specific rates were calculated by applying the method presented in section 2.4.2 (KNM method) from the data collected in 2004, 2005 and 2006. The other parameters of the matrix ***A*** were based on a literature review of similar natterjack populations across Europe (see section 2.5.2). It was not possible to determine the specific adult survival rates for the Irish population in the present study. This would require a mark-recapture study of the toadlet and adult populations to more accurately determine site-specific survival rates and age structure. The values chosen for the remaining elements (and the underlying assumptions and simplifications) of the matrix ***A*** are now described.

2.5.2 Estimation of the model parameters

Each projection starts immediately following the spawning period of the current year, *t*. The juveniles that survive through their first winter, until the beginning of the spawning period of year *t*+1, are denoted 'juveniles [1 winter]'. They are almost one year old.

All females greater than four years are considered adults and the adult female survival rate (P_4) is set at 0.85. This corresponds to the survival rate of adult females in the population breeding at Woolmer, England, calculated over the period 1988-1990 inclusive (Banks *et al.* . 1993, Denton & Beebee 1993). Further studies of populations in England did not reveal any detectable age-dependent mortality for either males or females (Denton & Beebee 1993).

G_1 is the survival of individuals from eggs to juveniles [1 winter]. It can be expressed as follows:

G_1 = S_e (survival of eggs to tadpoles) …
… x S_0 (survival of tadpoles to juveniles [0 winter]) …
…x S_1 (survival of juveniles [0 winter] to juveniles [1 winter]).

$$= S_e \times S_0 \times S_1 \quad \textbf{(8)}$$

where S is the survival from one stage to another, S_e and S_0 are the survival rates calculated in 2004 to 2006 for each site (global averages for each site). We have used site-specific projection matrices, so that, S_e and S_0 are here set as site specific average values obtained at each site over the three consecutive years studied.

Mark-recapture experiments on juveniles have been used in other studies to estimate the survival between metamorphosis and adulthood (noted $S_{m\text{-}a}$). In particular, this survival was estimated at 0.02 for a population in England studied between 1977 and 1990 (Banks & Beebee 1988, Banks *et al.* 1993), at 0.0061 for a population in Germany over the period 1991-1994 (Sinsch 1997), and at 0.0056 for a population in Spain over the period 1992-1994 (Tejedo *et al.* . 1997 in Sinsch 1997). We set $S_{m\text{-}a}$ at 0.05 in order to account for the likely underestimation of toadlet production in the present study. A value of 0.05 is not conservative, and is larger than recorded for other European populations. In setting the value of $S_{m\text{-}a}$ to 0.05 we minimise the chance of predicting population declines at sites where none exist (the value of $S_{m\text{-}a}$ has the third largest effect on population growth rate, see section 3.5.2; and is thus important in the context of predicting declines in Irish populations).

To define $S_{m\text{-}a}$ let us consider the following notations,

G_2 = survival of juveniles [1winter] to subadults [2 winters]
S_3 = survival of subadults [2 winters] to subadults [3 winters]

So,

$$S_{m\text{-}a} = S_1 \times G_2 \times S_3 \quad \textbf{(9)}$$

Survival among juveniles and young adults is likely to be lower than the survival of mature adults (Sinsch 1997, Stephan *et al.* . 2001). This is the case for species which produce many eggs of which very few survive to become adults, resulting in an increasing survival rate towards adulthood. This survivorship pattern is well documented and usually survival rates also become constant from a certain age (Begon *et al.* 1996). As a result, G_2 and S_3 are arbitrarily set at 0.4 and 0.7 respectively; these values are lower than the adult survival (0.85). S_1 can then be estimated as follows

$$S_1 = S_{m-a} / (G_2 \times S_3)$$

$$= 0.05 / (0.4 \times 0.7)$$

$$= 0.18$$

and G_1 can thus be calculated for each breeding site using equation (8).

G_3 represents the survival of subadults [3 winters] to the stage adult. For the purpose of the present study, we assumed that the annual survival rate was similar for any individual in stage 3, whatever its age. This survival rate S_3 is set at 0.7. We further assume that the population is stationary and that the age distribution in stage 3 is stable, as in Caswell (2001) and in Crouse *et al.* . (1987). It appears then that the relative proportion of subadults [2 winters] and subadults [3 winters] are 1 and S_3 respectively. Therefore,

$$P_3 = S_3 \times \text{proportion of subadults [2 winters]} = S_3 \times 1 / (1 + S_3)$$

and $$G_3 = S_3 \times \text{proportion of subadults [3 winters]} = S_3 \times S_3 / (1+ S_3)$$

and thus $P_3 = 0.41$ and $G_3 = 0.29$.

Lastly, two fertility terms appear in the matrix. F_4 represents the average number of female eggs produced in the year t+1 by each breeding female that survived from year t to t+1 and remained in the 'adult' stage. The average number of eggs produced per breeding female is noted e, the number of female eggs f. We assume a 1:1 sex ratio and therefore $f = 0.5 \times e$ female eggs are produced per female at each time step. Not all females breed each year and so the proportion of mature females that do breed each year is set at 0.65, in line with estimates from other European populations (e.g. Denton & Beebee 1993, Stephan *et al.* . 2001, Denton 1991). F_4 can thus be obtained as follows

$$F_4 = 0.5 \times e \times 0.65 \times P_4 \qquad \textbf{(10)}$$

$$= 0.27625 \times e.$$

Lastly, F_3 represents the average number of female eggs produced in year t+1 by each female who survived from the stage subadult [3 winters] to the stage adult [4 winters]. Previous studies have shown that young females tend to produce fewer eggs than older females (e.g. Banks & Beebee 1986). Consequently, we have set the average number of eggs produced by an individual young female at $0.5 \times e$ (and thus $0.5 \times 0.5 \times e$ female eggs). We further assume that only 20% of the subadult females breed successfully. F_3 can then be calculated as follows

$$F_3 = 0.5 \times 0.5 \times e \times 0.2 \times G_3 \qquad \textbf{(11)}$$

$$= 0.0145 \times e$$

Therefore the projection matrix used in the present study is

$$A = \begin{bmatrix} 0 & 0 & F_3 & F_4 \\ G_1 & 0 & 0 & 0 \\ 0 & 0.40 & 0.41 & 0 \\ 0 & 0 & 0.29 & 0.85 \end{bmatrix}$$

The only parameters that vary between sites are therefore G_1, F_3 and F_4. However at four sites (Fermoyle, Glenbeigh, Nambrackdarrig and Tullaree) empirical estimates of F_3 and F_4 are not available (it was not possible to assess the number of eggs in a sample of more than 3 egg strings), and thus the mean fertility at these four sites was set at e = 2,195 eggs (average fertility for the other seven sites representing the global fertility rate across Irish populations). We use these inter-site differences to make a limited exploration of the growth rates of the different toad populations (see section 3.5.).

In discrete time the model can be used to project the population size of each stage class into the future. The dominant eigenvalue λ of the matrix $\boldsymbol{A}$ gives the asymptotic population growth rate. Simply, this means that the population would grow at the rate λ if the present environmental conditions were maintained indefinitely (for details see Caswell 2001). The dominant eigenvalues were calculated for each breeding site using Matlab (Version 6.0.0.88 Release 12). A growth rate less than 1 indicates that a population is declining and is likely to go extinct unless environmental conditions improve. A growth rate greater than 1, indicates a growing population.

2.5.3 Sensitivity analysis

Sensitivity analyses have been used extensively in population and conservation ecology to identify which model parameters have the largest effect on population growth rate. Using the sensitivity analysis approach it is possible to identify the survival rate or fertility rate that will have the biggest effect on population growth rate. Conservation ecologists have used this technique to identify appropriate age or stage classes whose conservation will maximise the growth rate of endangered species populations (e.g. Stephan *et al.* . 2001, Crouse *et al.* . 1987). We apply this technique here to identify the parts of the toad life cycle that have the greatest affect on local population growth rates. This part of the life cycle can then be monitored and used as an indicator of the conservation status of the population.

The population growth rate λ is a function of all the entries a_{ij} of the projection matrix $\boldsymbol{A}$. Changes in any of these entries will change λ. A useful analysis is to examine how sensitive λ is to small changes in each of the matrix elements a_{ij}, whilst holding all others constant. The term s_{ij} is defined as the sensitivity of λ to a change in a_{ij}. Although in the present study some of the a_{ij} values are estimated from the literature, such a sensitivity analysis indicates how λ is likely to vary with the true value of the estimated a_{ij}. In addition, if sensitivities are particularly high for a given a_{ij}, it also indicates that this vital rate (a_{ij}) should be quantified as accurately as possible since it is likely to have a great effect on the population growth rate. The sensitivity of λ to each a_{ij} term can be calculated directly from the eigenvectors of the matrix $\boldsymbol{A}$.

$$s_{ij} = \frac{\partial \lambda}{\partial a_{ij}}$$

$$= \frac{v_i w_j}{(\mathrm{w}, v)}$$

where w_j is the j^{th} element of the right eigenvector $\boldsymbol{w}$ (stable stage distribution, defined as $\boldsymbol{Aw}= \lambda\boldsymbol{w}$), v_i is the i^{th} element of the left eigenvector $\boldsymbol{v}$ (reproductive value, i.e. the relative contribution of each stage to future generations, defined as $\boldsymbol{v}^T\boldsymbol{A}=\lambda\boldsymbol{v}^T$ where ^{T} denotes the transpose of $\boldsymbol{v}$), and $(\boldsymbol{w},\boldsymbol{v})$ describes a scalar product independent of i and j (Caswell 2001). Evaluating the sensitivity of the population growth rate to all entries in the matrix $\boldsymbol{A}$ allows for comparison of the relative importance of the various matrix elements (or life history parameters) for the population under study. The sensitivities s_{ij} measure the absolute sensitivity of λ to absolute changes in the matrix elements a_{ij}.

2.6 Statistical analyses

All statistical analyses were performed using the computer software SPSS (Version 11.0 for Windows). All data were checked for normality prior to analyses. Some variables were transformed (e.g. arcsine of survival rates, log + 1 of conductivity, square root of the water depth) to meet the assumptions of the tests (normality and homogeneity of variance). When data did not conform to normality or homogeneity of variance, non-parametric tests were performed. Pearson's product moment correlations were carried out when investigating the association between environmental variables and life history demographics. Paired *t*-tests (or the non parametric equivalent, e.g. Wilcoxon signed ranks tests) were used to compare two sets of estimates (e.g. survival rates) for each pond. One-way ANOVAs were performed to test for both the differences in the fertility between sites and across ponds within sites. Two-way ANOVAs without replication (or the non parametric equivalent, e.g. a Friedman test) were carried out to investigate the effect of two factors (e.g. Year and Site) on biotic and abiotic variables (e.g. number of egg strings, pH). Post hoc analyses (i.e. pair wise comparisons) were carried out after each ANOVA using the Tukey's test. Mean values are presented plus or minus the standard error.

In order to investigate the effects of explanatory environmental factors on biotic response variables (e.g. survival rates), we combined both continuous (e.g. pH) and categorical explanatory variables (e.g. pond permanency) using a Generalised Linear Model (GLM). GLMs permit a combination of ANOVA and regression analyses within a common statistical modelling framework (Grafen and Hails 2002). The regression model formula is of the form:

$$\mathrm{Y} = \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_n X_n + \varepsilon \qquad \textbf{(12)}$$

where Y is the response variable

X_i represents each of the explanatory variables (X_i can either be continuous or categorical)

β_i is the regression coefficient associated with variable X_i

ε is the error term (i.e. noise), it is drawn independently for each data point from a Normal distribution with mean 0 and variance σ^2 (σ^2 = unexplained variance of the dataset).

In the present study, we have investigated the effects of local environment on a number of response variables, for example, the number of egg strings (also expressed as a density of egg strings for each pond, see section 4.2), or the egg to toadlet survival rates.

For natterjack populations, survival rates tend towards small values and thus their distribution is strongly skewed (and survival rates can only take on values between 0 and 1). It is not appropriate, therefore, to assume that the data and residuals are normally distributed. Data transformation can be used to address this issue, however it does not avoid the problem of predicting negative survival rates from a fitted model. A common approach is to use a Poisson regression model, that is, a GLM with a Poisson error distribution and log link function (e.g. Zuur *et al.* 2006, Xiao 2004, Hawes *et al.* 2002). These analyses were performed using the software package Brodgar version 2.5.2 (Highland Statistics Ltd). The results of the analysis are not expressed as an ANOVA table, rather they are reported as the estimated coefficients, β_i, for each of the explanatory variables listed in (12) above, and their associated standard errors. The statistical significance of each coefficient is determined using a *t*-test (to the 5% significance level).

The aim of the statistical modelling used here is to find the optimal model that identifies the parameters that best explain the variability in the observed data (response variables). Zuur *et al.* . (2003) suggest that the Akaike Information Criterion (AIC) can be used to aid model selection. The AIC is a measure of goodness of fit. The AIC can be calculated for each possible combination of explanatory variables, and the model with the smallest AIC is chosen as the most optimal model (Zuur *et al.* 2006, Wu *et al.* 2006, Yen *et al.* 2004, Zuur & Pierce 2004, Zuur *et al.* 2003). We used a "backward" selection approach, where the initial model has all explanatory variables. Each explanatory variable is then removed in turn, and the new AIC calculated. The variable that gives the lowest AIC compared to the full model is then removed. The process is repeated with the remaining variables, until the AIC starts to increase.

Preliminary and exploratory analyses of the survival rate data showed that after initial model fit there was a pattern of increasing residual variation, indicating that the data was over dispersed (this means that the model fitted the data less well for the highest values of survival rate). To account for over dispersion, we introduced a dispersion parameter ϱ in the GLM. The resulting GLM is called a quasi-Poisson model (Tjur 1998, Lee & Nelder 2000, Zuur *et al.* 2006).

3. TOAD POPULATION DEMOGRAPHICS

3.1 Breeding activity and population sizes 2004-2006

From 2004 to 2006 each study site was initially surveyed during the first week of April. Each year at this period, egg strings were only observed at the sites located on the Iveragh Peninsula and at Roscullen Island (South of the dingle Peninsula), no tadpoles were present. At Lough Nambrackdarrig, natterjacks started spawning later towards the end of April and the beginning of May. In 2006 at Roscullen Island, egg strings were first observed around mid-March. On the North of the Dingle Peninsula (e.g. at the Maharees, Lough Gill, and Stradbally), breeding started later than at the most southern sites, around mid-April in all years.

Table 3.1 presents the total number of egg strings recorded in each pond at each site during the three years of survey, along with the corresponding estimates of the breeding population sizes. The table also shows the total number of egg strings for each of the subpopulations, that is, those located on the Dingle and Iveragh peninsulas separately. The table also provides an estimate for the total population size of natterjack toads in Co. Kerry. Whilst this latter figure is informative, we caution against its use as an indicator of the conservation status of the toad in Ireland. Many of the natterjack toads breeding ponds are isolated, and it is thus also important to consider the size of each isolated population.

Table 3.1: Total number of egg strings recorded at all sites (grouped according to possible metapopulations) in the 3 years of survey, and adult population sizes (see section 2.2.4 for estimation of population sizes). NF refers to pond that did not form and - to ponds that were not surveyed. For each site, the pond numbers correspond to those presented in Figs 7.1-7.9.

Breeding sites		**Number of egg strings**			**Estimated Population size**		
		2004	**2005**	**2006**	**2004**	**2005**	**2006**
Dingle Peninsula-North							
Fermoyle	Pond 1	0	0	0	0	0	0
	Pond 2	0	0	0	0	0	0
	Pond 3	3	0	0	9	0	0
	total	**3**	**0**	**0**	**9**	**0**	**0**
Stradbally	Pond 1	205	304	323	631	935	994
	Pond 2	159	256	258	489	788	794
	Pond 3	31	28	33	95	86	102
	Pond 4	85	168	225	262	517	692
	Pond 5	20	30	20	62	92	62
	Pond 6	13	41	18	40	126	55
	Pond 8	-	-	12	-	-	37
	Drains	60	41	103	185	126	317
	total	**573**	**868**	**992**	**1763**	**2671**	**3052**
L. Gill	NWshore	27	30	50	83	92	154
	Others	4	1	4	12	3	12
	total	**31**	**31**	**54**	**95**	**95**	**166**
Maharees	Pond 1	174	415	496	535	1277	1526
	Pond 4	NF	5	16	NF	15	49
	Pond 8	NF	25	48	NF	77	148
	Pond 9	NF	91	188	NF	280	578
	Pond 12	NF	6	43	NF	18	132
	Pond 15	NF	1	5	NF	3	15
	Pond 16	NF	14	10	NF	43	31
	Pond 23	23	323	250	71	994	769
	Pond 25	NF	72	73	NF	222	225
	total	**197**	**952**	**1129**	**606**	**2929**	**3474**
Tullaree	Pond 1	3	7	8	9	22	25
	Pond 2	6	19	30	18	58	92
	Pond 3	3	9	13	9	28	40
	total	**12**	**35**	**51**	**37**	**108**	**157**
Total		**816**	**1886**	**2226**	**2511**	**5803**	**6849**
Roscullen Island (Dingle Peninsula-South)							
	Area 1	77	413	800	237	1271	2462
	Area 2	14	119	73	43	366	225
	total	**91**	**532**	**873**	**280**	**1637**	**2686**

Table 3.1: continued

Breeding sites		Number of egg strings			Population size		
		2004	**2005**	**2006**	**2004**	**2005**	**2006**
Iveragh Peninsula-North							
L.Yganavan	Zone1	48	44	89	148	135	274
	Zone2	28	18	14	86	55	43
	Zone3	40	52	38	123	160	117
	Zone4	48	90	120	148	277	369
	Zone5	55	65	158	169	200	486
	total	**219**	**269**	**419**	**674**	**828**	**1289**
L.Nambrackdarrig		8	12	16	25	37	49
	total	**8**	**12**	**16**	**25**	**37**	**49**
Dooks	Pond 1	3	10	6	9	31	18
	Pond 2	12	30	11	37	92	34
	Pond 3	10	11	10	31	34	31
	Pond 4	2	6	31	6	18	95
	Pond 5	3	11	16	9	34	49
	Pond 6	15	500	135	46	1538	415
	total	**45**	**568**	**209**	**138**	**1748**	**643**
Glenbeigh	Quarry	24	15	40	74	46	123
	Field	28	51	14	86	157	43
	Marsh	-	1	1	-	3	3
	total	**52**	**67**	**55**	**160**	**206**	**169**
Total		**324**	**916**	**699**	**997**	**2818**	**2151**
Caherdaniel (Iveragh Peninsula-South)							
	Pond 1	24	135	114	74	415	351
	Pond 2	74	198	199	228	609	612
	total	**98**	**333**	**313**	**302**	**1025**	**963**
Combined Irish Populations		**1329**	**3667**	**4099**	**4089**	**11283**	**12612**

To investigate differences in the spawning activity between years, we used an unreplicated two-way Analysis of Variance (ANOVA) with "site" and "year" as main effects. A similar ANOVA using "year" and "pond" as main effects was also carried out at site level to investigate differences between years within site. It was necessary to use an unreplicated design because only a single measure for the total number of egg strings can be obtained for a site or a pond within a given year. A two-way ANOVA without replication avoids pseudoreplicating individual ponds within site, but allows for an evaluation of between year effects (and in this respect is equivalent to a paired *t*-test).

Over the three years of the survey the total number of egg strings recorded in Co. Kerry was in 2004: 1329, in 2005: 3667 and in 2006: 4099 egg strings. The two-way ANOVA using "year" and "site" as main effects indicates that there were significant differences between years in the amount of spawning activity ($F_{2,20}$ = 5.92, P<0.01). Breeding in 2004 was significantly lower than breeding in either 2005 or 2006 (Tukey's test: P = 0.013 and P = 0.006 respectively). In the same analysis the effect of site was also significant ($F_{10,20}$ = 7.70, P<0.001) indicating between-site differences in spawning activity (see Tables 3.1. and 3.3.). Because this analysis was unreplicated it was not possible to evaluate the interaction term between site and year. At the individual site level, differences between years occurred at the Maharees ($F_{2,16}$ = 35.184, P<0.001), Tullaree ($F_{2,4}$ = 38.472, P=0.002), and Dooks ($F_{2,10}$ = 4.629, P=0.038) (see Table 3.2). All these sites showed a significant increase in breeding activity between 2004 and 2005, and 2004 and 2006. No differences were observed at any sites between 2005 and 2006.

There were also important within site differences (see Tables 3.1 and 3.2). At Stradbally, Ponds 1, 2 and 4 were the most productive. At the Maharees, Ponds 1 and 23 were the most used by natterjacks. On the North of the Iveragh peninsula, Lough Yganavan (areas 1, 4 and 5) and Dooks (pond 6) were the most productive.

Table 3.2: P-values of the two-way ANOVAs without replication carried out for each site to investigate the differences in the spawning activity (number of egg strings) between three years of surveys. P_{pond} refers to the level of significance of the factor "pond", whilst P_{year} refers to the level of significance of the factor "year". NS refers to P-value showing no significant results. NT refers to cases where no test could be carried out (only 1 value).

Breeding sites	P-values	
	P_{pond} value	P_{year} value
Fermoyle	NS	NS
Stradbally	<0.001	NS
L.Gill	0.017	NS
Maharees	<0.001	<0.001
Tullaree	0.007	0.002
Roscullen Island	0.034	NS
L.Yganavan	0.013	NS
L.Nambrackdarrig	NT	NT
Dooks	0.014	0.038
Glenbeigh	0.041	NS
Caherdaniel	NS	NS

Over the three-year study period, most breeding activity took place at the Maharees and Stradbally, where egg string counts were the highest (see Table 3.3 and Figure 3.1). There was a large increase in the number of egg strings produced between 2004 and 2006 (in the Maharees the number of egg strings was six times higher in 2006 [n= 1129] than in 2004 [n= 197]). Each of these two sites contribute to more than 20% of the total breeding activity recorded at the 11 known breeding sites in Co. Kerry in 2005 and 2006 (approx. 24% for Stradbally in both years, and approx. 26% and 28% for the Maharees, in 2005 and 2006 (see Table 3.3)). In 2004, the Maharees only contributed to 15% of the breeding activity whilst Stradbally contributed to 43%. In 2004, breeding activity was lower than in 2005 and 2006 at the main ponds in the Maharees (Pond 1 and Pond 23), but also fewer pools formed during 2004, thus limiting the number of breeding ponds at these sites (7 ponds out of 9 did not form in 2004). At Roscullen Island the breeding activity also showed an increase over the three years of study (almost an order of magnitude increase between 2004 and 2006: n=91 and n=873 respectively), contributing to 21% in 2006 of the total breeding recorded in Co. Kerry, compared to just 7% in 2004.

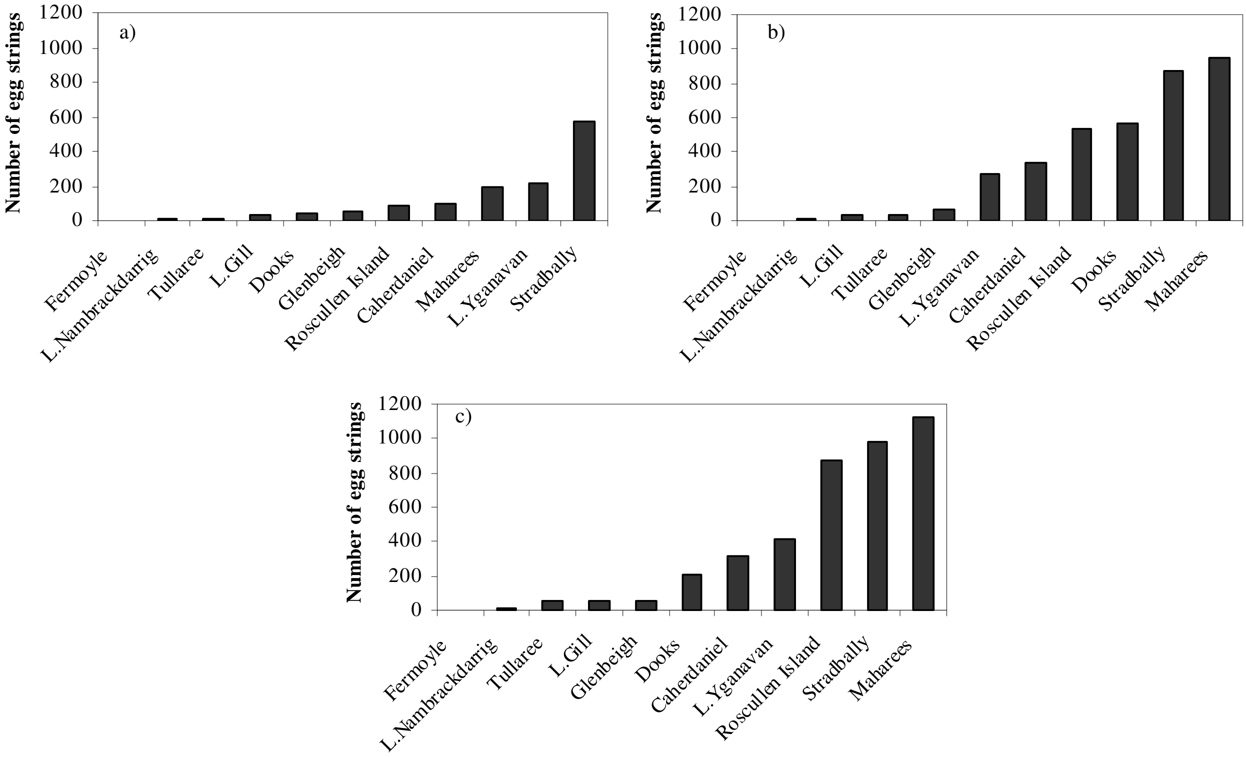


Figure 3.1: Total number of egg strings (y axis) recorded at each site for each of the three years of survey; a) 2004, b) 2005, c) 2006. Sites are ranked in ascending order.

Table 3.3: Number of egg strings recorded at each site for each year and contribution made to the breeding activity of the combined Irish natterjack populations.

	2004		2005		2006	
	Number of egg strings counted	**% of all breeding activity**	**Number of egg strings counted**	**% of all breeding activity**	**Number of egg strings counted**	**% of all breeding activity**
Dingle Peninsula-North						
Fermoyle	3	0.2	-	0	-	0
Stradbally	573	43.1	868	23.7	980	23.9
Lough Gill	31	2.3	31	0.9	54	1.3
Maharees	197	14.8	952	26.0	1129	27.5
Tullaree	12	0.9	35	0.9	51	1.2
Total	**816**	**61.3**	**1886**	**51.5**	**2214**	**53.9**
Roscullen Island (Dingle Peninsula-South)						
	91	**6.9**	**532**	**14.5**	**873**	**21.3**
Iveragh Peninsula-North						
L Yganavan	219	16.5	269	7.3	419	10.2
L Nambrackdarrig	8	0.6	12	0.3	16	0.4
Dooks	45	3.4	568	15.5	209	5.1
Glenbeigh	52	3.9	67	1.8	55	1.3
Total	**324**	**24.4**	**916**	**24.9**	**699**	**17**
Caherdaniel (Iveragh Peninsula-South)						
	98	**7.4**	**333**	**9.1**	**313**	**7.6**
Total	**1329**	**100**	**3667**	**100**	**4099**	**100**

At Dooks, the breeding activity was significantly lower in 2004 relative to 2005 and 2006 ($F_{2,10}$ = 4.629, P=0.038, see Table 3.2). Most of the egg strings were recorded at Pond 6, which is located in a pasture adjacent to the Dooks golf course, approximately 0.5 km away from the other five ponds in and beside the golf course. Pond 6 contributed to 88% (500/568) of the breeding activity at this site in 2005, and 65% (135/209) in 2006. The difference between 2004 and subsequent years is attributable to the fact that only the first meters at the border of the pond were surveyed in 2004, whereas, the whole surface area was monitored in the following years. The records in 2005 and 2006 are therefore more accurate and no population increase should be inferred between these two years and 2004.

In contrast to almost all other sites Fermoyle and Lough Nambrackdarrig showed very little breeding activity over the period 2004-2006. No breeding occurred at Fermoyle in 2005 and 2006. A combination of coastal erosion together with drainage of the land in the early 1980s led to the local extinction of the natterjacks at this site. New ponds were recently created to compensate for the previous loss. The size of the breeding population at this site was estimated at 9 individuals after three egg strings were recorded in 2004. No juvenile or adult toads were observed in 2005 and 2006. It is very possible that this local population has become extinct following very low recruitment in 2004.

Overall, the total number of natterjacks estimated to be present in Co. Kerry (based on the number of egg strings recorded) varied dramatically over the three years of study. Estimates in 2004 (a very dry year) suggested a total count of just 4,089 natterjacks (mature adults). In contrast, 2005 and 2006 counts are more consistent, providing estimates for the number of natterjacks across all sites of 11,283 and 12,612 individuals respectively. Our study indicates that there can be important variation in toad population sizes (4,000-12,000) among years. These estimates tally with previously suggested natterjack numbers in Ireland (ranging between 3,000 and 10,000 individuals for Co. Kerry, see McCarthy *et al.* , 1983; Beebee, 2002).

More than half the Co. Kerry natterjacks are present at the five sites on the North Dingle peninsula, possibly comprising a metapopulation. One quarter of the natterjack numbers are present on the North of the Iveragh peninsula, representing another possible metapopulation. Just 10% of the population occurs at Caherdaniel. The portion of the population represented at Roscullen Island increased from 7% in 2004 to 21% in 2006.

3.2 Double clutching

Although studies have shown that double clutching in natterjack toads was rare (in the UK, see Denton 1991, and in Sweden, see Silverin & Andren 1992), a recent study by Denton and Beebee (1996) reported that in a British natterjack population of approximately 40 breeding females, double clutching occurred in all four years studied (1992-1995). In this relatively small population, between 5% and 28% of the females laid a second clutch of eggs, with on average 63 days between clutch depositions (with a minimum of 58 days). Consequently, for the Irish populations surveyed in 2004, 2005 and 2006, we assumed that the egg strings laid 58 days or more after the date where eggs strings were first recorded could be second clutches. This corresponds to a total of 120, 229 and 142 possible double clutches in 2004, 2005 and 2006, which only represent 9%, 6% and 3% of the total number of egg strings recorded each year (see Table 3.4).

Table 3.4: Potential number of egg strings representing double clutches (n) and corresponding proportion (%) relative to the total number of egg strings recorded at each site in 2004, 2005 and 2006.

Site	2004		2005		2006	
	n	%	n	%	n	%
Stradbally	52	9	66	8	38	4
Maharees	15	9	26	3	1	0.2
Tullaree	0	0	2	6	0	0
Roscullen	18	24	4	1	31	4
Yganavan	7	3	28	10	19	5
Dooks	12	39	17	3	18	9
Glenbeigh	10	20	0	0	6	17
Caherdaniel	16	16	86	35	29	9
Total	120	9	229	6	142	3

From Tables 3.1 and 3.4, it is possible to investigate whether the much higher number of eggs recorded in 2005 and 2006 compared to 2004 can be due to a higher proportion of double clutches in 2005 and 2006. To do so, we excluded Dooks field (Pond 6) since the area surveyed in 2005 and 2006 was larger than in 2004. When subtracting the potential double clutches in 2005 and 2006 from the total number of egg strings recorded in 2005 and 2006 (respectively 3,167 and 3,964 strings excluding Dooks field), this still leads to a much higher number of strings than recorded in 2004 (1,314 strings).

Even when potential double clutches are taken into account, the number of egg strings recorded in 2005 and 2006 are still more than twice as high as in 2004. On a site by site basis breeding in 2004 either occurred (ponds formed) or it did not (ponds did not form). We are unable to accurately determine what proportion of the female breeding population at each site contributed to the breeding in each year of the study. Our population estimates for each site and each year are based on the assumption that 65% of all females have visited a pond and laid an egg string. If the proportion of females that contributed to the breeding activity was lower in 2004 (due to dry conditions) and higher in 2005 and 2006, it is possible that the size of the toad population was underestimated in 2004 and overestimated in 2005 and 2006. This suggests the importance of inter annual variability of weather conditions in the determination of population size.

3.3 Fertility of the Irish populations in 2006

3.3.1 Fertility specific to each Irish population

To estimate the mean number of eggs per string, a sample of 222 fresh egg strings (formed of two rows of eggs) were chosen randomly, throughout the 2006-breeding season at eight different sites. No egg strings could be sampled at Fermoyle, Tullaree and Glenbeigh due to the absence of breeding activity (Fermoyle) or the presence of abundant vegetation which did not enable us to sample egg strings without damaging them.

On average, the method used to count the number of eggs (using the software ImageJ) led to an over-estimation of 55 eggs (see Table 3.5), however this difference is not significant at the 5% level (paired *t*-test, P=0.369).

Table 3.5: Differences in the number of eggs per string estimated using ImageJ and counted manually on the printed photograhs. The difference is not significant (NS) at the 5% level (paired *t*-test, P=0.369).

Estimated number of eggs using ImageJ	Counted number of eggs (manually)	Difference "estimated"-"counted"
627	635	-8
1543	1475	68
1549	1586	-37
1324	1271	53
2746	2274	472
2825	2697	128
3583	2840	743
2851	2608	243
4240	3777	463
2080	2289	-209
1368	1372	-4
2934	2902	32
3687	3990	-303
1346	1414	-68
2338	2463	-125
1143	1287	-144
947	1011	-64
1994	2123	-129
2446	2512	-66
Average difference		**55**^{NS}

Based on the 222 egg strings analysed, a female natterjack lays on average 2,195 eggs in a string (see Table 3.6). There were significant differences between sites (One-way ANOVA, $F_{6,211}$ = 22.876, P<0.001). Egg strings laid at Stradbally (n=1329 egg per strings) appeared to be smaller than at the other sites (see Fig. 3.2 and Table 3.6). The difference was significant for the Maharees (n=3189 eggs per strings), Caherdaniel (n=2790) and Dooks (n=2114) (Tukey's test: P<0.001, see Table B in Appendix 2). In contrast, in the Maharees, females natterjack tended to lay longer egg strings. The difference was significant with Stradbally and Lough Yganavan (n=1765 eggs per strings) (Tukey's test: P<0.001, see Table B in Appendix 2). Within sites, One-way ANOVAs did not show any significant differences between ponds.

Table 3.6: Mean number of eggs per string specific to each pond and site (where egg strings could be photographed and processed). The number of egg strings analysed at each pond and the standard error of the mean are also provided.

Breeding sites		**Mean number of eggs per string**	**Number of egg strings studied**	**SE**
Stradbally	Pond 1	1363	17	180
	Pond 2	1184	16	95
	Pond 4	1431	25	125
	Pond 7	1171	5	177
	total mean	**1329**	**63**	**75**
L.Gill	NW shore	1938	3	344
	total mean	**1938**	**3**	**344**
Maharees	Pond 1	3071	25	209
	Pond 4	2318	1	-
	Pond 9	3772	3	984
	Pond 23	3327	5	577
	Pond 25	3417	6	252
	total mean	**3189**	**40**	**166**
Roscullen Island	Area 1	2380	3	620
	total mean	**2380**	**3**	**620**
L.Yganavan	Zone1	2250	15	514
	Zone2	1377	1	-
	Zone3	2338	1	-
	Zone4	1791	14	256
	Zone5	1245	15	101
	total mean	**1765**	**46**	**194**
L.Nambrackdarrig		2617	1	-
	total mean	**2617**	**1**	-
Dooks	Pond 1	2367	1	-
	Pond 2	2584	5	360
	Pond 3	2028	2	418
	Pond 4	1475	2	377
	Pond 5	2409	4	710
	Pond 6	2346	1	-
	total mean	**2114**	**19**	**233**
Caherdaniel	Pond 1	3175	25	297
	Pond 2	2420	26	192
	total mean	**2790**	**51**	**182**
Mean for Co. Kerry populations		**2195**	**222**	**85**

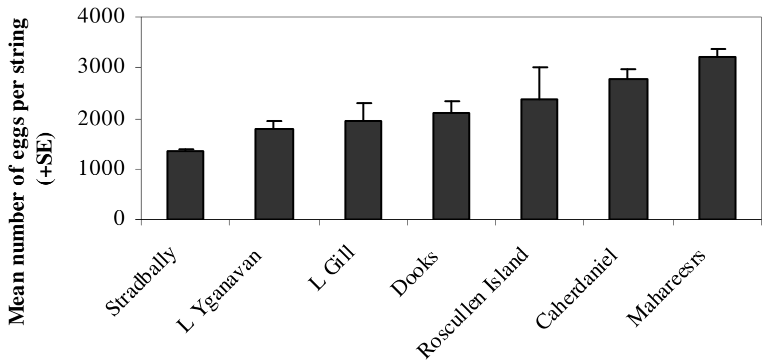


Figure 3.2: Estimated mean number of eggs per string and standard error bars for each study site.

3.3.2 *Comparison of fertility values with other European populations*

Table 3.7 presents the average clutch size for natterjack toad populations at 7 different European countries, including the global average clutch size for the Irish populations. Although based on different sample sizes, our results suggest that female fertility for Irish natterjack populations is lower than most other European populations where fertility levels have been assessed using similar methods (egg counts in fresh egg strings). This is not surprising, considering that the Irish populations represent the north western limit of the species distribution.

Table 3.7. Natterjack toad clutch sizes recorded within the distribution range of the species in Europe. The average fertility is shown as well as the total number of egg strings (n) for which eggs were counted in each study.

Average fertility	n	Habitat	Country	Authors
2,195 (SE=85)	222	Sand dunes, marshes and permanent lakes	Ireland	Bécart *et al.* 2006 (present study)
1,839 (SE=198)	11	Sand pit	Belgium	Stevens *et al.* . 2003
2,045 (SE=170)	21	Sand pit	Belgium	Stevens *et al.* . 2003
2,894 (SE=147)	37	Sand dunes	Britain	Banks & Beebee 1986
4,975 (SE=366)	22	Heathland	Britain	Banks & Beebee 1986
3,000-4,000	-	-	Britain	Smith 1951 in Beebee 1979
3,000-7,000	-	-	Spain	Salvador 1974 in Beebee 1979
3,819 (SE=128)	115	Ephemeral ponds	Spain	Tejedo 1992
3,600	32	-	Germany	Kadel 1975
3,000-4,000	-	-	Germany	Mertens 1964 in Beebee 1979
3,200- 4,000	-	-	Poland	Kowalewski 1967 in Beebee 1979
3,000-4,000	-	-	Sweden	Curry-Lindhal 1975 in Beebee 1979

3.4 Breeding success 2004-2006

3.4.1 Validating the KNM survival rate method

3.4.1.1 Comparison between two methods for the calculation of survival rates

At Stradbally, it was possible to distinguish between the newly metamorphosed toadlets and the older toadlets, in particular at Ponds 1 and 2. At these two ponds tadpole densities were high, and therefore recently metamorphosed and emerged toadlets were still very small and completely black. After only a few days, they quickly became larger and changed coloration. At Stradbally, Pond 1, newly emerged toadlets represented 45% of the total number of toadlets estimated on each visit in 2006, and this was also the case in 2005 (44%) but not in 2004 (76%). We were thus able to estimate the total production of toadlets for this pond based on the cumulative counts of newly emerged toadlets throughout the different breeding seasons. Table 3.8 presents the results obtained when calculating the egg to toadlet survival rate using the KNM method and the ratio toadlets:eggs.

Table 3.8: Comparison between egg to toadlet survival rates at Strabally, Ponds 1 and 2, using the KNM method and the ratio toadlets:eggs. The fertility was set at 2,000 eggs.

Pond		1			2		
Year		2004	2005	2006	2004	2005	2006
Egg to toadlets survival (%)	Toadlets:eggs	0.6	0.9	0.8	0.2	1.4	3.9
	KNM method	0.5	1.1	0.9	0.3	1.3	3.6
Difference		- 0.1	0.3	0.1	0.1	- 0.1	- 0.3

From Table 3.8, it is apparent that the survival rates calculated using the KNM method did not differ significantly from the survival rates calculated using the crude ratio toadlets:eggs (Wilcoxon signed ranks test, $P = 0.914$). However, the toadlets:eggs ratio is only usable at sites or ponds where there is a good knowledge of the total production of toadlets (and hence why it has not been used throughout the present study).

Table 3.9 presents a similar comparison for one pond (Maharees, Pond 23), where most of the toadlets emerged simultaneously (during the same week). Simultaneous emergence provides a good estimate of the total production of toadlets. We present the same analysis at two additional ponds at Tullaree (Ponds 2 and 3). At Tullaree, because the vegetation surrounding the ponds is so high (hummocks, tussocks), it is only possible to survey the first metre immediately around the ponds for toadlets. In addition, the number of egg strings laid (breeding activity) at Tullaree was much lower than at the Maharees, Pond 23 and at Stradbally, Ponds 1 and 2. The survival rate calculated using both methods appear similar for the Maharees, Pond 23 but not for Tullaree, Ponds 2 and 3 (Table 3.9). These results therefore suggest that the calculation of the survival rate (whatever the method used) is not accurate for small populations such as the one breeding in Tullaree. In the absence of any other information, it is not possible to state which of the two methods most accurately estimates the true survival rate.

Table 3.9: Comparison between egg to toadlet survival rates at Maharees, Pond 23, and Tullaree, Ponds 2 and 3, using the KNM method and the ratio toadlets:eggs. The fertility was set at 2,000 eggs per string.

Pond		Maharees Pond 23	Tullaree Pond 2	Tullaree Pond 3
Year		2006	2006	2006
Egg to toadlets survival (%)	Toadlets:eggs	5.3	4.5	11.7
	KNM method	5.2	2.6	6.2
Difference		- 0.1	- 1.9	- 5.5

3.4.1.2 Sensitivity of the KNM method to fertility values

In order to investigate the sensitivity of the KNM method to changes in the values chosen for the fertility rates (eggs per string), we explored the relationship between the estimated survival rates and fertility. To do this we varied the fertility values from 500 to 3000 eggs for each of 7 ponds (selected on the basis that some tadpoles survived to the toadlet stage), and using just the data collected in 2006. Survival rate declines logarithmically with increasing fertility rate. This logarithmic decline in survival is consistent across each of the seven ponds studied (see Table 3.10 which lists regressions describing this logarithmic decline). For brevity, just three of these relationships are presented in Figure 3.3.

Table 3.10: Relationship between egg to toadlet survival and fertility for 7 ponds where some tadpoles survived to metamorphosis. Survival rates are the ones obtained in 2006 and are expressed as proportions (<1). Each regression has 3 degrees of freedom. The regression is of the type: $survival = a + (b \times \ln(fertility))$.

Site	Pond	R^2	P	a	b
Stradbally	1	0.99	0.001	0.0864	-0.0102
Stradbally	2	0.99	<0.001	0.2966	-0.0341
Stradbally	4	0.85	0.025	0.0103	-0.0012
Maharees	1	0.90	0.014	0.0072	-0.0008
Caherdaniel	1	0.85	0.025	0.0061	-0.0006
Roscullen	1	0.99	<0.001	0.2895	-0.0337
Yganavan	1	0.95	0.005	0.0016	-0.0002

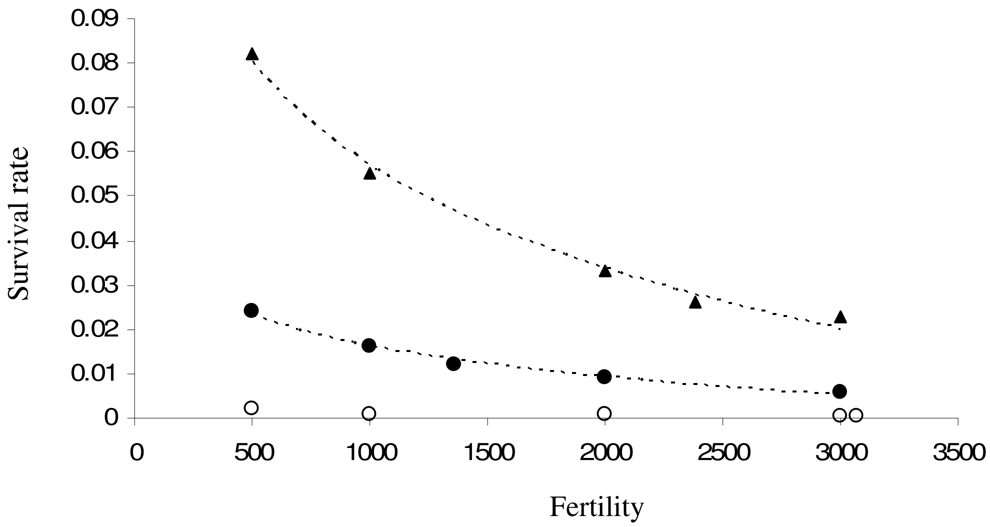
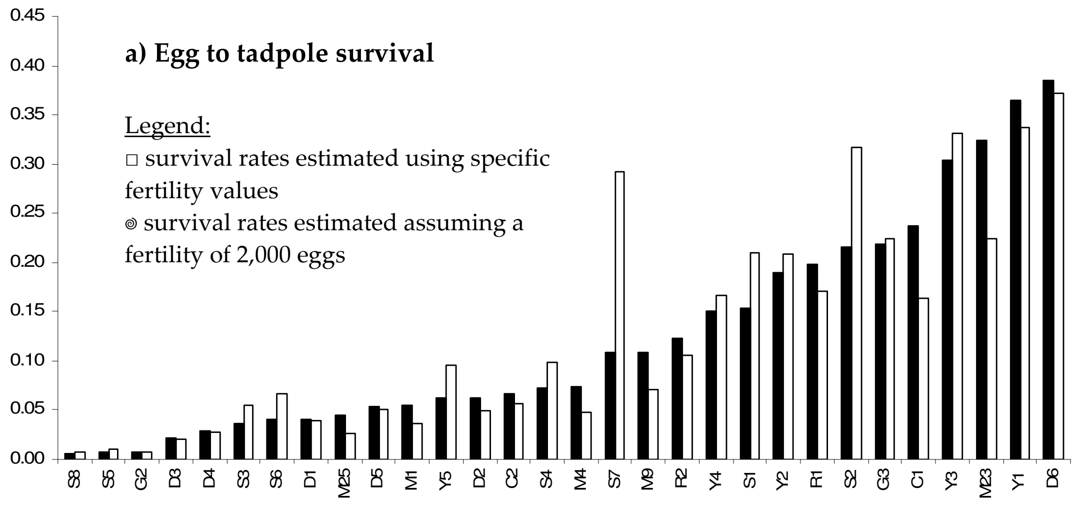


Figure 3.3: Relationship between egg to toadlet survival rate (proportion) and fertility values for Roscullen, Area 1 (▲), Stradbally, Pond 1 (●) and Maharees, Pond 1 (O). The dotted lines represent the logarithmic regression lines fitted to the simulated data.

Our aim here is not to describe the functional regression relationship between fertility and survival (the two are auto-correlated); rather we wish to understand how survival behaves as a function of the fertility rate. The analysis indicates that the KNM method is more sensitive to fertility values lower than 2,000 eggs than to fertility values greater than 2,000 eggs. The survival rates calculated for ponds, where toads have a low fertility, should thus be considered with care, as these survival rates are likely to represent an important underestimation of survival compared to sites where fertility values are above 2,000 eggs.

We might therefore expect differences when ranking the ponds according to natterjack toad survival rates, when these are calculated using the empirically measured fertility values as opposed to a fixed value of 2,000 eggs (Aubry & Emmerson, 2004; and approx. global average of 2,195). Figure 3.4 presents the estimated survival rates for the egg to tadpole and egg to toadlet stages obtained for ponds where there were non zero survival rates, and for which some egg strings had been sampled in the field and used to estimate the fertility values.



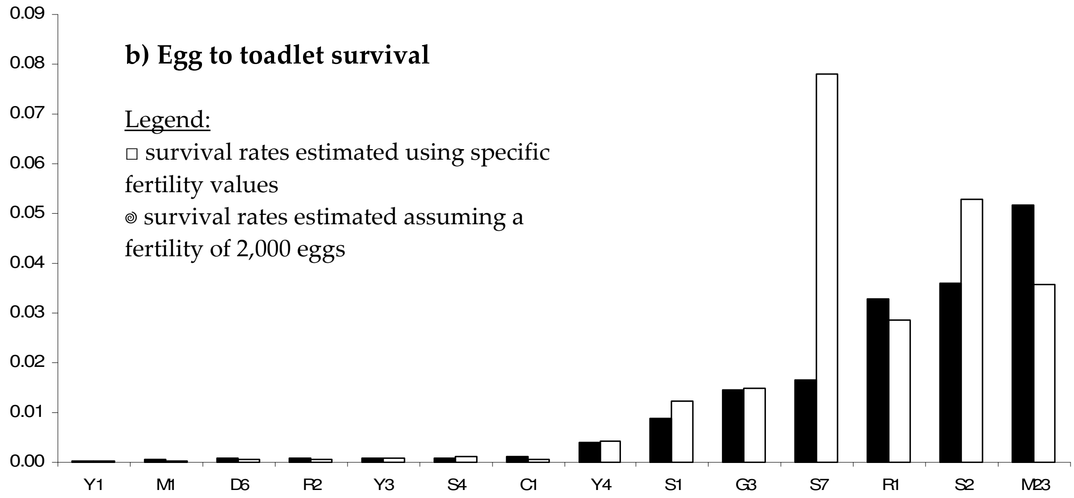


Figure 3.4: a) Egg to tadpole survival rates and b) egg to toadlet survival rates, calculated using the KNM method, assuming a fertility of 2,000 eggs (black bars) or a fertility estimated using field samples of eggs strings (white bars). Each pond is represented by the first letter of the site and the number of the pond within the site. The survival rates were calculated using the data collected in 2006.

Both the egg survival rate and egg to toadlet survival rate were underestimated at Stradbally, Ponds 2 and 7 (Figure 3.4 a) and b). This is because the fertility estimated in these two ponds was among the lowest (1,184 and 1,171 eggs respectively, see Table 3.6). On the contrary, survival rates were overestimated at ponds with relatively high fertility values, such as Caherdaniel, Pond 1 and the Maharees, Pond 23 (3,175 and 3,327 eggs respectively, see Table 3.6). Although the ranking of the ponds according to their survival rates differed between the two methods (see Figure 3.4), the difference between the two estimations of survival rates was non significant (Wilcoxon signed ranks test, P = 0.984 and P = 0.594 for egg survival and egg to toadlet survival respectively). This result indicates that estimating the survival rates using a fertility value of 2,000 eggs for each pond does not lead to a consistent over or underestimation. In other words, the survival rate at a pond for which there is no information on the fertility is as likely to be underestimated as to be overestimated. At 4 of the 11 studied sites, we do not have an accurate estimation of the mean number of eggs per strings, survival rates at these ponds were calculated using a fixed value of 2,195 eggs per string (which represents the global mean for Irish populations).

3.4.1.3 Testing the validity of the KNM method by estimating the duration of each stage

At most sites, an accurate estimation of the total number of eggs produced is available. We can therefore obtain an estimate of the duration of each stage. As mentioned in the methods section, the estimated values of the stage durations are simply a form of KNM model validation.

The duration of the egg and tadpole stages were estimated for the 17 ponds where some tadpoles did metamorphose in 2006 (i.e. for ponds where A_2 and $A_3 \neq 0$ so that the equation (2) in section 2.4.2 can be calculated). The results obtained are consistent with the ecology of the natterjack toads, with an average of 18.6 ± 1.3 days for the egg stage and 51.1 ± 8.1 days for the tadpole stage.

Overall, the investigations of the sensitivity and validity of the KNM method indicate that this method is robust and is an appropriate method for the estimation of natterjack toad egg and larval survival rates.

3.4.2 Breeding success of each toad population

Table 3.11 presents the egg and tadpole survival rates estimated for each breeding pond in each year (2004 to 2006). In 2006 Tullaree and Roscullen Island appeared to be the most successful populations, with egg to toadlet survival rates at 3.0 % and 2.6 % respectively. The least successful were the most southern populations, in particular Caherdaniel, Glenbeigh, Dooks and Nambrackdarrig, where the egg to toadlet survival rates were below 0.1 %. Tadpole survival was much lower than egg survival for all populations in all years except in the Maharees where in 2006 tadpole survival (10.22 %) was higher than the egg survival (8.11 %). This is because in 2006 the egg survival in the Maharees was exceptionally low following the early drying of 7 of the 9 pools that were used by the toads to breed.

Table 3.11: Survival rates of each stage estimated for the 3 annual surveys (2004 - 2006). Survival rates are expressed as %. NF refers to ponds that did not form, 0^{PD} to ponds where the survival rate was zero because the pond dried out, NSp when no spawning occurred, and "-" when the pond was not surveyed.

Breeding sites		Egg survival (%)			Tadpole survival (%)			Egg to toadlet survival (%)		
		2004	**2005**	**2006**	**2004**	**2005**	**2006**	**2004**	**2005**	**2006**
Fermoyle	Pond 1	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp
	Pond 2	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp	NSp
	Pond 3	55.4	NSp	NSp	0.54	NSp	NSp	0.30	NSp	NSp
	mean	**55.4**	**NSp**	**NSp**	**0.54**	**NSp**	**NSp**	**0.30**	**NSp**	**NSp**
Stradbally	Pond 1	38.3	40.1	21.4	1.64	3.67	5.83	0.63	1.47	1.25
	Pond 2	31.3	28.4	29.3	1.47	6.44	16.7	0.46	1.83	4.88
	Pond 3	5.35	2.38	5.43	0	0	0	0	0	0
	Pond 4	13.1	31.0	10.5	0.45	0.55	1.26	0.06	0.17	0.13
	Pond 5	3.20	0.63	1.03	0	0	0	0	0	0
	Pond 6	42.8	4.98	5.97	0.16	0.03	0	0.07	0	0
	Pond 8	-	-	0.76	-	-	0	-	-	0
	Drains	2.01	6.65	15.5	0^{PD}	0^{PD}	15.2	0^{PD}	0^{PD}	2.35
	mean	**25.9**	**29.0**	**18.9**	**1.40**	**3.74**	**10.3**	**0.36**	**1.09**	**1.95**
Lough Gill	NW shore	40.3	29.2	22.4	4.81	7.26	6.62	1.94	2.12	1.48
	Other areas	12.2	0	0.79	0	0	0	0	0	0
	mean	**36.6**	**28.2**	**20.8**	**4.60**	**7.26**	**6.60**	**1.69**	**2.05**	**1.37**
Maharees	Pond 1	0.29	3.68	3.54	0.27	0.26	0.93	0^{PD}	0.01	0.03
	Pond 4	NF	0^{PD}	4.74	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}
	Pond 8	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}
	Pond 9	NF	0^{PD}	7.13	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}
	Pond 12	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}
	Pond 15	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}
	Pond 16	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}
	Pond 23	2.22	1.94	23.1	0^{PD}	0.56	15.9	0^{PD}	0.01	3.68
	Pond 25	NF	0^{PD}	2.84	NF	0^{PD}	0^{PD}	NF	0^{PD}	0^{PD}
	mean	**0.51**	**2.26**	**8.11**	**0.13**	**0.35**	**10.2**	**0^{PD}**	**0.01**	**0.83**
Tullaree	Pond 1	23.1	28.9	43.9	1.18	3.06	0.88	0.27	0.89	0.39
	Pond 2	33.7	40.4	33.0	0.02	0.15	7.30	0.01	0.06	2.41
	Pond 3	53.8	51.7	56.6	0.29	1.03	10.5	0.15	0.53	5.96
	mean	**36.1**	**41.0**	**40.7**	**0.30**	**0.85**	**7.36**	**0.11**	**0.35**	**3.00**

Table 3.11: continued

Breeding sites		Egg survival (%)			Tadpole survival (%)			Egg to toadlet survival (%)		
		2004	**2005**	**2006**	**2004**	**2005**	**2006**	**2004**	**2005**	**2006**
Roscullen Island	Area 1	50.3	30.6	17.1	3.85	5.75	16.7	1.93	1.76	2.85
	Area 2	7.37	3.59	10.5	0[PD]	4.99	0.66	0[PD]	0.18	0.07
	mean	**43.7**	**24.6**	**16.6**	**3.75**	**5.72**	**15.8**	**1.64**	**1.41**	**2.62**
Lough Yganavan	Zone1	28.6	2.73	39.4	11.1	0.33	0.09	3.16	0.01	0.04
	Zone2	13.9	2.62	20.9	2.11	0	0	0.29	0	0
	Zone3	39.3	19.4	33.2	3.25	5.46	0.28	1.28	1.06	0.09
	Zone4	33.4	19.6	16.7	1.08	10.6	2.58	0.36	2.07	0.43
	Zone5	26.1	7.20	6.94	1.69	0.11	0	0.44	0.01	0
	mean	**29.2**	**12.7**	**19.5**	**3.92**	**10.3**	**0.71**	**1.14**	**0.90**	**0.14**
Nambrackdarrig		**31.1**	**21.3**	**62.8**	**0.10**	**0.01**	**0.15**	**0.03**	**0.002**	**0.09**
Dooks	Pond 1	8.19	2.38	4.07	0[PD]	0	0	0[PD]	0	0
	Pond 2	7.18	2.61	6.24	0[PD]	0	0	0[PD]	0	0
	Pond 3	3.80	0.77	2.16	0[PD]	0	0	0[PD]	0	0
	Pond 4	3.27	2.41	2.96	0[PD]	0	0	0[PD]	0	0
	Pond 5	9.91	11.3	5.35	0[PD]	0	0	0[PD]	0	0
	Pond 6	56.6	19.3	38.5	2.49	0.30	0.19	1.41	0.06	0.07
	mean	**23.0**	**17.4**	**26.3**	**2.05**	**0.30**	**0.18**	**0.47**	**0.05**	**0.05**
Glenbeigh	Quarry	0.45	11.3	39.7	0[PD]	0[PD]	0.17	0[PD]	0[PD]	0.07
	Field	46.8	11.4	33.6	0.06	0.09	0.01	0.03	0	0
	Marsh	-	-	22.4	-	-	0.75	-	-	0.17
	mean	**25.4**	**11.2**	**37.8**	**0.06**	**0.07**	**0.14**	**0.02**	**0.01**	**0.05**
Caherdaniel	Pond 1	21.5	10.4	18.2	4.49	0.05	0.43	0.97	0.01	0.08
	Pond 2	9.54	8.64	4.89	0.02	0.09	0	0	0.01	0
	mean	**12.5**	**9.35**	**9.75**	**1.91**	**0.07**	**0.29**	**0.24**	**0.01**	**0.03**

To investigate differences in the breeding success between years and sites, and within sites, we performed Friedman tests due to the non-normality of survival rate data. This test is a non parametric analogue of a 2-way ANOVA. When considering the mean survival rates for each site, the three survival rate measures differed among sites (egg to tadpole: P = 0.030; tadpole to toadlet: P = 0.019; and overall egg to toadlet: P = 0.023). In contrast, there were no significant differences among years.

Within sites and years, we were only able to detect differences in the survival rates of eggs at Dooks (P = 0.030), where egg survival in 2005 was lower than in 2004 and 2006 (see Table 3.11). At all the other sites there were no significant differences in survival of eggs or tadpoles between years. Lastly, we were able to detect significant differences between ponds at the two sites where more than 5 ponds were used for breeding (Stradbally, P = 0.028 and P = 0.033 for the tadpole survival and egg to toadlet survival respectively; and at Dooks, P = 0.024, P = 0.010 and P = 0.010 for the egg survival, tadpole survival and egg to toadlet survival respectively). At the remaining two sites (Tullaree and Lough Yganavan) where more than 2 ponds or breeding areas had been used each year (and thus where the Friedman test could be carried out), there were no significant differences among ponds or breeding areas.

These results indicate that there are important differences in the egg and tadpole survival rates among sites and also within sites. Unlike the number of egg strings (and thus breeding activity), the survival rates (and thus breeding success) at each site remain relatively constant through time (2004-2006) (see section 2.5).

3.5 Population growth analysis

3.5.1 Growth rates for each natterjack population

The dominant eigenvalues were calculated for each population matrix and are presented in Table 3.12. Each growth rate was calculated using the average egg to toadlet survival rates obtained at each site over the three consecutive years studied. The site-specific fertility values estimated in 2006 were used to calculate the egg and tadpole survival rates, and to obtain the fertility parameters F_3 and F_4 in the population matrix.

Table 3.12. Population growth rates (λ) for each natterjack toad population. The adult population size was estimated using the number of egg strings calculated in 2006.

Breeding site	λ (growth rate)	Adult population size (estimate)
Roscullen Island	1.1386	2686
Lough Gill	1.0934	166
Tullaree	1.0583	157
Stradbally	1.0008	3015
Lough Yganavan	0.9856	1289
Maharees	0.9553	3474
Fermoyle	0.9340	0
Dooks	0.9071	643
Caherdaniel	0.8890	963
Nambrackdarrig	0.8719	49
Glenbeigh	0.8597	169

The net growth rates of the populations at Roscullen Island, Lough Gill and Tullaree are greater than 1, which indicates that if the current environmental conditions remain constant these populations will continue to grow (until they reach the carrying capacity of the habitat). This is because tadpole survival rates at these three sites are the highest relative to the other eight sites (see Table 3.11). However the populations at Tullaree and Lough Gill are much smaller than at Roscullen (low 100s) and are thus more vulnerable to chance events, e.g. land use change. The growth rates of the populations at the remaining sites are either very close to or lower than 1, which may indicate that these populations are at equilibrium (i.e. at carrying capacity), for example Stradbally, or they may be in decline. In particular, the mean population growth rate of Fermoyle, the Maharees, Nambrackdarrig, Dooks, Glenbeigh and Caherdaniel (average growth rate 0.9028 ± 0.015) is significantly different from 1 (t-test, $P = 0.001$) indicating that these populations are almost certainly in decline.

However, we offer a note of caution with regard to these population growth rates. The growth rates listed in Table 3.12 are based on the projection matrix $\boldsymbol{A}$, and most of its elements are derived from published data measured from other European populations. Consequently, in the absence of further population parameters specific to the Irish populations (in particular the adult survival rates), there is a degree of uncertainty over these predicted population declines. Our estimation of these demographic rates has been on the generous side so that population declines should not occur due to these estimations; rather population declines can be attributed to poor survival of tadpoles. The present approach does enable us to compare growth rates between sites, and it is possible to identify the populations that are the most vulnerable to the risk of extinction if breeding success remains poor in the next 10 or 20 years.

When the assessment of population growth rate is considered together with the estimates of population size (small populations are inherently vulnerable to extinction) we see that a number of populations are at risk of local extinction. It appears that the Glenbeigh, Lough Nambrackdarrig and Fermoyle sites are the most vulnerable populations (see Table 3.12), due to their relatively low population size and growth rates. The populations at Dooks and

Caherdaniel also have a low growth rate, indicating that there is a need to implement conservation measures at these sites. The population growth rates at the Maharees, Stradbally and Lough Yganavan are very close to or slightly below one, maybe indicating populations at or close to carrying capacity. The population size at each of these sites is large (low 1000s), and so values for $\lambda<1$ might not necessarily indicate that the populations are in decline.

Another way to assess whether populations are likely to be declining is to observe the average age of the adult toads at the breeding ponds. If these data were available over a period of several years, a senescent and isolated population would show an increase in the average age of the adults breeding at the ponds (indicating a high proportion of old breeders). However, to assess the age of the adults on a regular basis is not possible, and requires invasive techniques for aging (i.e. removal of toes for aging in the lab).

3.5.2 *Sensitivity analysis*

The sensitivity of the growth rate to the survival rates and fertility values are presented in Table 3.13 for each of the toad populations.

Table 3.13. Sensitivity (s_{ij}) of the population growth rates to survival rates and fertility levels for each natterjack toad population. The survival rates (G_1, G_2, G_3, P_3 and P_4) and fertility values (F_3 and F_4) correspond to the population parameters of the stage-classified model presented in Figure 2.2. The survival rates G1 were estimated using the average egg to toadlet survival rates obtained at each site over the three consecutive years studied. The fertility parameters (F3 and F4) are based on the site specific fertility estimated in 2006, except for Fermoyle, Tullaree and Glenbeigh for which F3 and F4 are similar and based on an average of 2,195 eggs per string.

Population	Sensitivity of λ to the population parameters						
	G_1	G_2	G_3	P_3	P_4	F_3	F_4
Fermoyle	117.9	1.1585	0.2154	0.1210	0.7432	0.00003	0.0001
Stradbally	48.32	0.2464	0.3309	0.1668	0.6362	0.0001	0.0003
Lough Gill	45.12	0.3459	0.4569	0.2024	0.5445	0.0002	0.0002
The Maharees	150.1	0.1887	0.2554	0.1384	0.7036	0.00003	0.0001
Tullaree	59.89	0.3102	0.4123	0.1914	0.5741	0.0001	0.0002
Roscullen Island	45.84	0.3893	0.5103	0.2137	0.5128	0.0002	0.0002
Lough Yganavan	69.72	0.2280	0.3069	0.1584	0.6565	0.0001	0.0002
L. Nambrackdarrig	183.9	0.0501	0.0688	0.0434	0.9106	0.000002	0.00003
Dooks	136.0	0.1160	0.1583	0.0933	0.8044	0.00002	0.0001
Glenbeigh	203.3	0.0234	0.0322	0.0208	0.9575	0.000001	0.00002
Caherdaniel	204.6	0.0839	0.1149	0.0701	0.8544	0.00001	0.00004

From Table 3.13, it is apparent that the growth rate λ of each population is most sensitive to G_1, i.e. the survival rate of eggs, tadpoles and toadlets. This sensitivity analysis does not allow the assessment of the specific sensitivities of λ to the egg and tadpole survival rates, because G_1 integrates these measures. On the contrary, fertility values of the adults (F_3 and F_4) have only a very small influence on population growth rates. Although, fertility values are also incorporated into the estimates of G_1 via the initial numbers of eggs laid in ponds. The second parameter to which λ is the most sensitive (after G_1) is the adult survival rate (P_4). Further exploration of the model shows that G_1 consistently has the largest effect on growth rate even when the empirically measured survival rate G_1 is increased by two orders of magnitude.

These findings suggest that increased survival in the first year will generally result in a higher population growth rate, and thus in a larger number of reproductive adults. This corresponds to previous studies where it was shown that population size of pond breeding amphibians is largely determined by the survival of the larval stages in the pond (Alford & Richards 1999, Semlitsch 2002, Wilbur 1980 and 1997). Conservation efforts should therefore concentrate on increasing the survival of the toad larval and juvenile stages (especially juveniles in their first year), as this will have the most significant effect on population size.

3.6 Population sizes and risk assessment

Based on the estimates of population sizes, survival rates and population growth rates of Irish natterjack populations over the period 2004-2006, we can group the populations into four main categories:

i) Small populations at risk (< 50 adults , λ <1): Fermoyle, Nambrackdarrig.
ii) Medium populations at risk (< 1000 adults, λ <1): Glenbeigh, Dooks, Caherdaniel.
iii) Medium populations (< 1000 adults, λ ≥1): Lough Gill, Tullaree.
iv) Large populations (> 1000 adults, λ ≥1): Lough Yganavan, Roscullen Island, Stradbally, the Maharees.

3.7 Conclusion: Toad life cycle stage suggested for use as an indicator of conservation status

The important annual variability in toad breeding activity indicates that it is essential to record the number of egg strings over consecutive years (at least 3). Such an approach is especially important when assessing the conservation status of amphibians such as *B. calamita*, which breed in temporary ponds subject to environmental fluctuations. Trends in the number of egg strings over several breeding periods can then provide information about changes in population size. It is important to highlight the important dynamism of the species. With such species breeding in temporary ponds, some years will face complete breeding failure whereas more favourable years (e.g. more rainfall) will lead to a large number of recruits into the existing toad population. This will then lead to the arrival of young breeders 3-4 years later, and this pattern will further contribute to the interannual variability in population size and breeding activity.

Another alternative could be to base the assessment of population size on adult counts during night surveys. We recommend the use of egg string counts for two main reasons. First, although toads are likely to come to the ponds to breed under specific weather conditions (typically when the air temperature is warm (approx. 10ºC) and when there is little wind), this is not always the case. We have observed toads calling on cold (below 8ºC) and very windy nights, whereas on some occasions when the conditions appeared optimal for toad breeding, very few adults were observed at the ponds. Also, for the surveys to be comparable among sites would require each site to be surveyed during the same nights, which would require many surveyors. Second, on any given night, it is not possible to estimate accurately the proportion of the males that are coming to breed at the pond. This proportion is likely to change during the season, among sites and even overnight (e.g. it has been shown that young males tend to come at the ponds later in the season than the older males and that some individuals tend to always breed earlier than others, see Beebee 1979, Sinsch 1997). It is thus difficult to estimate population sizes using adult counts.

The findings of the present study further suggest that increased survival in the first year will generally result in a higher population growth rate, and thus in a larger number of reproductive adults. Conservation efforts should therefore concentrate on assessing and increasing the survival of the toad tadpole and juvenile stages (especially juveniles in their first year), as this will have the most significant effect on population size. It is often difficult to assess the survival of juvenile toads, and so survival rates of the toad aquatic stages can be assessed when there is a good knowledge of (i) the total number of eggs laid in the pond and of (ii) toadlet production (at least to an order of magnitude). Knowledge of toadlet production is particularly important as it also gives an immediate indication of the site viability: failure to produce toadlets for 2 or 3 years probably indicates that a serious problem has arisen (HCT 2003). In such instances, this information obtained still provides a few years before the population is likely to become extinct. In that time remedial measures can be taken to improve the conditions at the site.

It is therefore important to use both the number of egg strings laid each year and the production of toadlets as an indicator of the species conservation status. Whatever the method used to estimate breeding success (egg to toadlet survival rate), it is likely to represent an underestimation, since the total number of toadlets can rarely be assessed accurately.

4. ENVIRONMENTAL FACTORS AFFECTING THE SPECIES CONSERVATION STATUS

Several environmental factors (e.g. pH, conductivity, water temperature, and water depth) were measured at all ponds across the 11 study sites and throughout the 2004 to 2006 breeding seasons. These measures are reported in Tables C, D and E in Appendix 3. Our aim here is to investigate the effects of these environmental factors on the breeding activity and survival rates of natterjack toads. We have used General Linear Models (GLMs) in which each response variable (egg density and survival rates) was expressed as a function of the monitored environmental factors (i.e. explanatory variables) (e.g.: pH, conductivity, for continuous variables, habitat, pond permanency, for nominal variables). The computer software Brodgar (Version 2.5.2) was used to carry out the analyses.

4.1 Environmental parameters over the three-years surveyed and investigation of differences between sites

For each environmental parameter measured (pH, conductivity, water temperature, water depth), we compared the mean value for the breeding season at each site between the three years of study. Figure 4.1 shows how these environmental parameters varied over the three years of study. Two-way ANOVAs without replication (with factors "year" and "site" used as main effects) indicated that there were no significant differences between the three years surveyed for pH, conductivity and water temperature (see Table 4.1). There were significant differences in water depth between years (see Table 4.1). These same ANOVAs did show significant site differences for each of the environmental parameters (see Table 4.1).

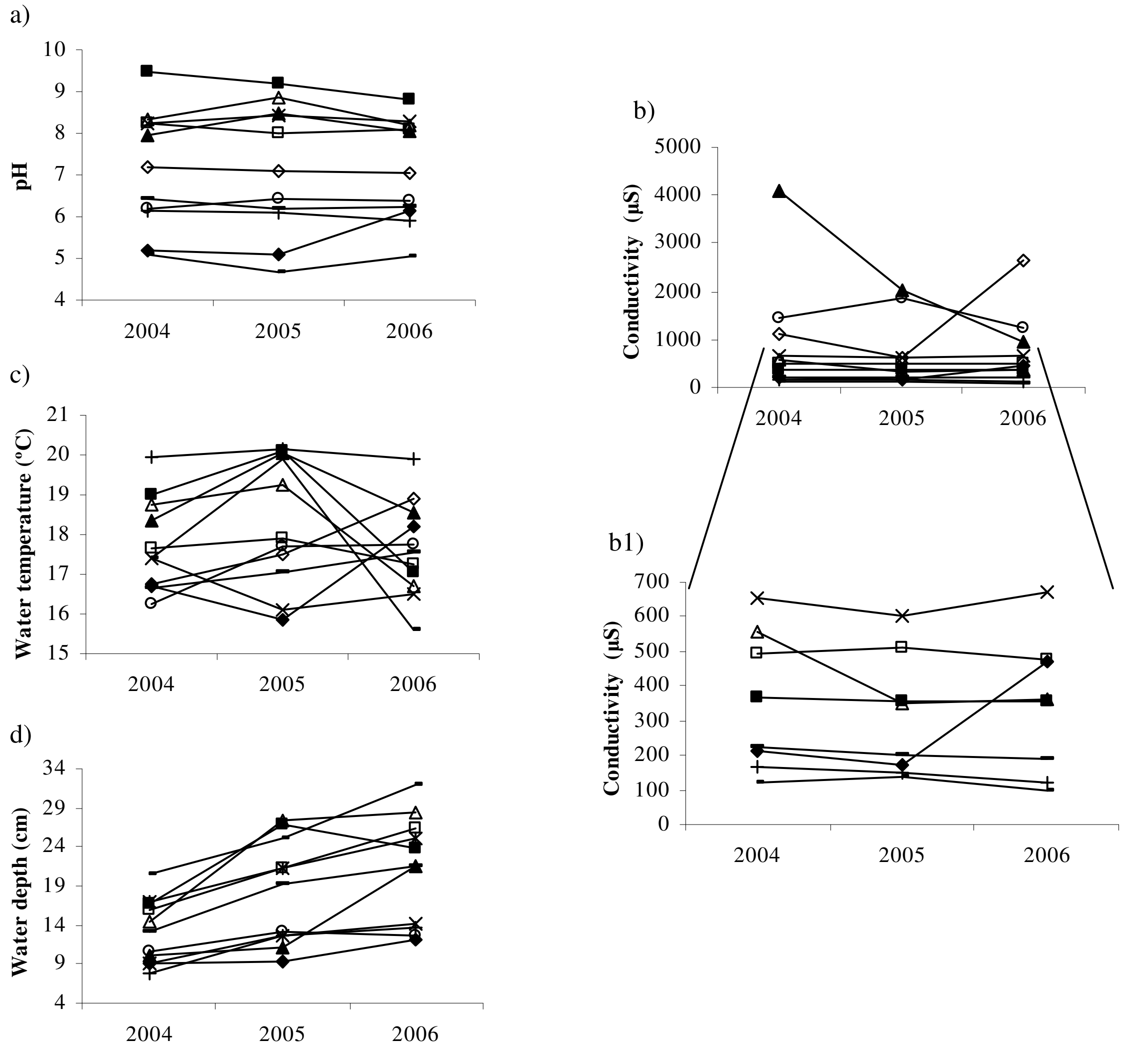


Figure 4.1 : Evolution of pH (a)), conductivity (b)), water temperature (c)) and water depth (d)) over the three-year survey. The values presented are the mean values for the breeding season. ∆ represents Fermoyle, □ Stradbally, ▲ Lough gill, × the Maharees, ◊ Tullaree, + Lough Yganavan, - Lough Nambrackdarrig, — Dooks, ♦ Glenbeigh, and ■ Caherdaniel. b1) shows an enlargement of the lower values of conductivity. The corresponding standard errors are not shown here (for reasons of clarity), they are listed in Tables C, D and E in Appendix 3.

Table 4.1: Results of the two-way ANOVAs without replication performed on pH, conductivity water temperature and water depth (average values of each year at each site). The ANOVAs were performed with the factors "year" and "site" as main effects.

Environmental parameters	F-statistic	P-value
pH		
Year	$F_{2,20}=0.014$	0.986
Site	$F_{10,20} = 68.229$	<0.001
Conductivity		
Year	$F_{2,20}= 0.572$	0.574
Site	$F_{10,20} = 18.201$	<0.001
Water temperature		
Year	$F_{2,20}= 1.290$	0.297
Site	$F_{10,20} = 2.453$	0.042
Water depth		
Year	$F_{2,20}= 3.962$	<0.001
Site	$F_{10,20} = 17.416$	<0.001

4.1.1 pH

The pH varied significantly between sites. Fermoyle, Stradbally, Lough Gill and the Maharees had a similar pH (ranging on average from 7.9 to 8.8, throughout the years (see Appendix 3). These four sites formed a homogenous subset and were all significantly different from the other breeding sites (Tukey's test: $P \leq 0.05$ for all four sites except Fermoyle, which showed no difference with Caherdaniel [see Table F in Appendix 4 for a detailed breakdown of these site differences]). Over the three-year study, pH at Caherdaniel was significantly higher (on average from 8.8 to 9.5 than at the other sites (apart from Fermoyle) (Tukey's test: $P<0.05$ for all cases). In contrast, Lough Nambrackdarrig had the lowest pH throughout the three-year study (Tukey's test: $P \leq 0.01$ for all pairwise comparisons except with Glenbeigh). Glenbeigh had the second lowest pH over the course of the study.

4.1.2 Conductivity

Conductivity was also significantly different between sites (see Figure 4.1 and Table 4.1). Lough Gill, Tullaree and Roscullen Island had the highest conductivity (on average close to 1000 $\mu S.cm^{-1}$ up to over 4000 $\mu S.cm^{-1}$) over the three years. In particular, Tukey's test showed significant differences between Lough Gill and all the other sites (apart from Tullaree and Roscullen Island, $P<0.05$ for all concerned pairwise comparisons [see Table G in Appendix 4]). For Tullaree and Roscullen Island, significant differences appeared mainly with the Southern sites (sites on the Iveragh Peninsula). These three sites all have a marine influence via connections to the sea (e.g. drains) explaining the rise in conductivity compared to the other sites. The conductivity at Lough Nambrackdarrig and Lough Yganavan are the lowest across our study region.

4.1.3 Water temperature

Over the study period, average water temperatures ranged from 16ºC to 20ºC and were much less variable between sites than the other environmental variables (see Table 4.1, P=0.042). Natterjack toad breeding sites are all broadly located within the same region (Co. Kerry) and are thus very likely to be affected in the same way by local regional climate.

4.1.4 Water depth

All of the ponds where toads were breeding were relatively shallow, with an breeding average water depth ranging from 9 to 32 cm (see Appendix 3). However, there were significant differences in mean water depth amongst sites (see Table 4.1). Glenbeigh, Lough Yganavan, the Maharees, Roscullen Island and Lough Gill had significantly shallower waters than the rest of the sites (Tuckey's test P<0.05 for all pairwise comparisons except with Dooks, see Table H in Appendix 4). Fermoyle and Lough Nambrackdarrig were the deepest ponds/breeding areas.

4.1.5 Correlations among environmental variables

Prior to the statistical analyses, we explored the explanatory environmental variables for any possible correlation to check for co-linearity. These correlations are shown in Table 4.2.

Table 4.2: Correlation parameters among environmental variables and associated P-values. The complete three years data set was used apart for salinity/conductivity correlation, where only the data with a salinity value different from zero were used. R_s refers to the Spearman's rho correlation coefficient. R_P refers to the Pearson correlation parameter. Cd refers to conductivity and PP to Pond Permanency. The table only represents correlation coefficients higher than 0.4 (or below -0.4).

	Correlation parameter	**P-value**
pH vs Cd (log)	R_P=0.45	<0.001
Salinity vs Cd	R_P=0.90	<0.001
PP vs Activity	R_s=-0.52	<0.001
Water Depth vs PP	R_s=-0.49	<0.001

It is apparent from Table 4.2 that pH and Conductivity, Activity and Pond Permanency and Pond Permanency and Water Depth are significantly correlated (respectively, r = 0.45, r = -0.52 and r = -0.49, P<0.001 in all cases). Consequently, if two correlated variables appear significant in the following analyses (GLMs), the final model will only consider the one with the higher explanatory power. Conductivity and Salinity are highly correlated (r = 0.90, P < 0.001). We therefore excluded Salinity from the analyses.

4.1.6 Consideration of the factors "site" and "habitat" in the GLM

The results presented above indicate that a large proportion of the variability amongst the environmental parameters is explained by the factor "site" (Table 4.1.). In sections 3.1, 3.3, 3.4, we also show that the biotic parameters such as breeding activity and survival rates are highly dependent on the factor "site". In fact, it is the combination of the different environmental parameters that constitute each site, and thus accounting for the differences between sites. These parameters make a site favourable or not in terms of breeding activity or survival. Toads interact with their environment at local scales, they do not interact with "site". Therefore, including the factor "site" in the GLM obscures the effects of the continuous explanatory variables (which are significantly different across sites). Because the factor "site" has a significant effect on the other continuous explanatory variables (e.g. pH, conductivity, water depth and water temperature), we have chosen to remove site from the analysis. We know that these continuous explanatory variables account for the differences between sites, and thus, we use them to account for differences in breeding activity at different breeding ponds.

Similarly, we can also remove the factor "habitat" from the model. Each of the environmental parameters measured are likely to be specific to a given habitat. For instance, a low pH and conductivity are expected from bogs or heathlands. Two-way ANOVAs run for each of the environmental parameters using "year" and "habitat" as main effects showed a significant effect of the factor "habitat" on pH, conductivity and water temperature (see Table 4.3). Although water temperature does not vary with habitat type, the remaining continuous explanatory variables are affected by this factor. We have therefore removed the factor "habitat" from the model.

Table 4.3: Results of the two-way ANOVAs without replication performed on pH, conductivity, water temperature and water depth means for each year. The ANOVAs are performed with "habitat" and "year" as main effects, using the average values obtained for each habitat type.

Environmental parameters	F-statistic	P-value
pH		
Year	$F_{2,8}$= 1.457	0.29
Habitat	$F_{4,8}$= 541.5	<0.001
Conductivity		
Year	$F_{2,8}$= 1.196	0.35
Habitat	$F_{4,8}$= 35.9	<0.001
Water temperature		
Year	$F_{2,8}$= 0.375	0.70
Habitat	$F_{4,8}$= 2.5	0.13
Water depth		
Year	$F_{2,8}$= 36.86	<0.001
Habitat	$F_{4,8}$= 6.948	0.01

4.2 Environmental factors and breeding activity

The size of breeding ponds varies across the study sites, therefore breeding activity (number of egg strings) is not directly comparable between ponds. The number of egg strings recorded may be dependent on the size of the area surveyed (i.e. an area effect of sampling larger habitats, for example, Tullaree vs. Yganavan). To gain an estimate of breeding intensity we have used the density of egg strings (per metre of shoreline), rather than an overall egg string count for each site. The perimeter of some ponds (in particular in the Maharees) is highly variable through time, and so we have set the value for the perimeter of each pond as the average perimeter in April (based on 2004, 2005 and 2006 measurements).

Table 4.4 presents the results of the GLM using egg density as the response variable and environmental parameters presented in section 4.1 as explanatory variables. The stepwise removal of explanatory variables shows that the best fitting model for egg density includes year, pH and water temperature (see Table 4.4). The estimated regression coefficients for the factors "Year2" and "Year3" were highly significant ($\gamma_{3,2}$ = 1.0486 and $\gamma_{3,3}$ = 1.2508, P<0.01 see Table 4.4), indicating that the density of eggs in 2005 (year 2) and 2006 (year 3) was significantly higher than in 2004. The first year of survey (2004) was drier than the following years and fewer ponds were available for natterjack breeding.

The effect of water temperature on the density of egg strings is also highly significant (γ_2 = 0.1525, P<0.05, see Table 4.4). Thus, warmer ponds are favoured for spawning by natterjacks. Finally, the factor "pH" was also significant (γ_1 = 0.3382, P < 0.01, see Table 4.4), indicating that the density of eggs tended to be higher in ponds with higher pH values.

Table 4.4: Results of the GLM analysis using the density of egg strings as a response variable. The table presents the estimated GLM coefficients for each factor with associated standard error, the z-value and significance level. The model assumed a Poisson error distribution (with a log link function). *** refers to P<0.001, ** refers to P<0.01, * refers to P<0.05.

NB: Initial model: log (egg-density) = α' + β_1 pH + β_2 Conductivity + β_3 Water Temperature + β_4 Water Depth + $\beta_{5,i}$ Year$_i$ + $\beta_{6,i}$ Activity$_i$ + $\beta_{7,i}$ Pond Permanency$_i$; AIC= 230.51; Overdispersion (Deviance/df.residual) = 1.02.

Final model: log (egg-density) = α + γ_1 pH + γ_2 Water Temperature + $\gamma_{3,i}$Year$_i$; AIC= 224.16; Overdispersion (Deviance/df.residual) = 1.02.

	Estimate	Std. Error	z value	Pr(>|z|)	Degree of significance
(Intercept)	α = -6.8193	1.7251	-3.953	<0.001	***
pH	γ_1 = 0.3382	0.1100	3.076	0.0021	**
Water Temperature	γ_2 = 0.1525	0.0718	2.124	0.0337	*
Year2	$\gamma_{3,2}$ = 1.0486	0.3841	2.730	0.0063	**
Year3	$\gamma_{3,3}$ = 1.2508	0.3848	3.251	0.0012	**

4.3 Environmental factors and survival rates

As for the density of egg strings, we used the same explanatory variables to explore the variability in egg to tadpole survival and egg to toadlet survival rates between ponds and sites. The data for egg to tadpole and egg to toadlet survival were over dispersed and to correct for this in the GLM we applied a quasi Poisson model with a dispersion parameter ϱ (see methods section 2.6).

4.3.1 Egg to tadpole survival

The results of the GLM using egg to tadpole survival as the response variable are presented in Table 4.5. The stepwise removal of explanatory variables shows that the best fitting model includes Pond permanency, Year, Water temperature, Conductivity and pH. Conductivity and pH are correlated (see Table 4.2) and consequently, we have removed pH from the model (of the two, pH had the least explanatory power). The final model thus includes Pond permanency, Year, Water temperature and Conductivity (see Table 4.5). The estimated regression coefficient for Year2 (i.e. 2005) was significant ($\gamma_{3,2}$ = -0.57230, $P < 0.05$, see Table 4.5), indicating that the egg to tadpole survival was lower in 2005 than in 2004. There was no difference in the egg to tadpole survival rates between 2004 and 2006.

Water temperature had a significant positive effect on egg to tadpole survival rates (γ_2 = 0.1530, $P < 0.01$, see Table 4.5), indicating that the probability of survival from egg to tadpole was higher in warmer ponds. Such an observation is consistent with our current knowledge of natterjack egg development requirements. Eggs cannot hatch when temperatures are below 10°C and their development is enhanced at warmer temperatures (Beebee 1983). Furthermore, in cold waters eggs are vulnerable to *Saprolegnia* infection (Banks and Beebee 1988). Increased conductivity also had a significant positive effect on egg to tadpole survival rates (γ_1 = 0.44206, $P < 0.05$, see Table 4.5).

Finally, the factor pond permanency also had a significant effect on egg to tadpole survival. In particular, the regression coefficient for the factor "Pond Permanency 3" (corresponding to ephemeral ponds, that dried out before the end of the breeding season) was significantly different from the other measures of permanency (e.g. permament and semi permanent) ($\gamma_{4,3}$ = -0.55713, $P < 0.05$, see Table 4.5). These differences indicate that egg to tadpole survival is significantly lower in ephemeral ponds than in more permanent ponds. Such a result is unusual because natterjacks are known to preferentially utilize ephemeral ponds for breeding. The hydroperiod of the pond is therefore an important cause of egg mortality. In 2006 in particular, 4 of the 9 pools used in the Maharees by natterjacks dried out before any eggs could hatch and no tadpoles were observed in these ponds.

Table 4.5 : Results of the GLM analysis using the egg to tadpole survival as a response variable. The table presents the estimated GLM coefficients for each factor with associated standard error, the *t*-statistics and corresponding significant level. The model assumed a quasi-Poisson distribution (with a log link function). *** refers to P<0.001, ** refers to P<0.01, * refers to P<0.05.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**	**Degree of significance**
(Intercept)	α = -0.59153	1.16001	-0.510	0.6112	NS
Conductivity	γ_1 = 0.44206	0.21656	2.050	0.0430	*
Water temperature	γ_2 = 0.15300	0.04929	3.104	0.0025	**
Year2	$\gamma_{3,2}$ = -0.57230	0.21997	-2.602	0.0107	*
Year3	$\gamma_{3,3}$ = -0.23018	0.19711	-1.168	0.2457	NS
Pond permanency2	$\gamma_{4,2}$ = -0.35720	0.21449	-1.665	0.0990	NS
Pond permanency3	$\gamma_{4,3}$ = -0.55713	0.23909	-2.330	0.0218	*

NB: Initial model: log (egg survival) = $\alpha' + \beta_1$ pH + β_2 Conductivity + β_3 Water Temperature + β_4 Water Depth + $\beta_{5,i}$ $Year_i$ + $\beta_{6,i}$ $Activity_i$ + $\beta_{7,i}$ Pond $Permanency_i$; Over dispersion (Deviance/df.residual): ϱ = 10.8; AIC= 1695.

Final model: log (egg survival) = $\alpha + \gamma_1$ Conductivity + γ_2 Water temperature + $\gamma_{3,i}$ $Year_i$ + $\gamma_{4,i}$ Pond $Permanency_i$; Over dispersion (Deviance/df.residual): ϱ = 12.7; AIC= 1875.

4.3.2 *Egg to toadlet survival*

The results of the GLM using egg to toadlet survival as the response variable are presented in Table 4.6. The stepwise removal of explanatory variables shows that the best fitting model includes Conductivity, Water temperature, Pond permanency and Year. The estimated coefficients for the factors Year and Pond permanency were not significantly different from zero (see Table 4.6), suggesting that there were no differences in the egg to toadlet survival among the three years studied and the three states of permanency of the ponds. It is however important to keep the factor Year in the final model, in order to reflect the fact that the same ponds are surveyed each year and cannot be considered as replicates. The factor Pond permanency was also kept in the model (based on the AIC), which suggests that the status of the pond is likely to have an effect on the egg to toadlet survival, despite the failure of the model to provide regression coefficients significantly different from zero.

Conductivity had a positive effect on the egg to toadlet survival (γ_1 = 1.63839, P<0.001, see Table 4.6). Eggs laid in a pond with a relatively high conductivity will thus tend to develop more successfully into toadlets than eggs laid in ponds with a lower conductivity. This is likely to reflect positive effects of higher nutrient levels on food availability. Water temperature also had a significant positive effect on egg to toadlet survival (γ_2 = 0.33912, P < 0.05, see Table 4.6), again consistent with our current knowledge of the optimum conditions for egg and tadpole development (see above, section 4.3.1). In warmer waters tadpoles are more active (personal observations) and may be able to avoid predation more effectively.

Table 4.6 : Results of the GLM analysis using the egg to toadlet survival as a response variable. The table presents the estimated GLM coefficients for each factor with associated standard error, the *t*-

statistics and corresponding significant level. The model asumed a quasi-Poisson distribution (with a log link function). *** refers to P<0.001, ** refers to P<0.01, * refers to P<0.05.

NB: Initial model: log (egg to toadlet survival) = $\alpha' + \beta_1$ pH + β_2 Conductivity + β_3 Water Temperature + β_4 Water Depth + $\beta_{5,i}$ $Year_i$ + $\beta_{6,i}$ $Activity_i$ + $\beta_{7,i}$ Pond $Permanency_i$; Over dispersion parameter: $\varrho = 1.36$; AIC= 182.6

Final model: log (egg to toadlet survival) = $\alpha + \gamma_1$ Conductivity + γ_2 Temperature + $\gamma_{3,i}$ $Year_i$ + $\gamma_{4,i}$ Pond

	Estimate	Std. Error	t value	Pr(>|t|)	Degree of significance
(Intercept)	$\alpha = -11.48612$	2.90799	-3.950	<0.001	***
Conductivity	$\gamma_1 = 1.63839$	0.45940	3.566	<0.001	***
Water Temperature	$\gamma_2 = 0.33912$	0.13386	2.533	0.0133	*
Year2	$\gamma_{3,2} = -0.07165$	0.59525	-0.120	0.905	NS
Year3	$\gamma_{3,3} = 0.67128$	0.51614	1.301	0.197	NS
Pond_permanency2	$\gamma_{4,2}= -0.79662$	0.51139	-1.558	0.123	NS
Pond_permanency3	$\gamma_{4,3} = 0.11407$	0.55796	0.204	0.839	NS

$Permanency_i$; Over dispersion parameter: $\varrho = 1.32$; AIC= 178.56

4.4 Conclusion: Principal environmental parameters influencing natterjack breeding activity and success

The investigation of the environmental variables that best account for variation in the response variables (i.e.: egg density, egg to tadpole, and egg to toadlet survival rates) led to consistent conclusions. In particular, water temperature had a significant positive effect on both breeding activity and success. This is consistent with our knowledge of natterjack developmental biology (see above section 4.3.1). Consequently, ponds should be managed to maximise mean temperature, whilst maintaining hydroperiod. Therefore to maximise reproduction and development, this means that ponds should have relatively shallow margins, with little or no shade.

The permanency of a pond appears to have a significant influence on egg to tadpole, and egg to toadlet survival. The most ephemeral ponds (i.e. those that tend to dry out before the end of the breeding season) tend to have much lower survival of eggs and tadpoles. These ponds tend to be very shallow, but also very warm, thus attracting natterjacks. In summary, it seems that there is a fine balance to achieve between pond depth, temperature and hydroperiod (ephemerality), allowing regular recruitment and occasional drying out thus limiting the abundance of tadpole predators. This aspect is being more precisely investigated (Bécart *et al.* in prep).

Conductivity had a significant positive effect on the survival of eggs to tadpoles and eggs to toadlets, whereas pH had a significant positive effect on the intensity of breeding activity. Conductivity and pH are correlated (see Table 4.2), and it appears therefore that they both have an effect on the natterjack toad breeding activity and success, and thus on the species conservation status.

5. PRACTICAL MONITORING PROTOCOL FOR THE SPECIES

In this section, we provide some recommendations regarding the future surveys of natterjack toad breeding ponds (suitable for implementation by NPWS rangers). The aim of the following monitoring protocol is to assess the conservation status of the Irish natterjack toad populations. Ideally, and depending on the funds available, it would be preferable to employ a full time surveyor for a four months period each year (April – July), which would allow a complete assessment of the population size and breeding success at all the sites in Co. Kerry. Natterjack toads have a particularly extended breeding season, and unless each site is visited intensively (each 8 to 10 days) during the breeding season, it is not possible to obtain an accurate estimate of each toad population size and level of breeding success. However, a less intense (and thus less time demanding) but well focused monitoring program could provide some useful insights into the conservation status of the different toad populations. We provide below some guidelines regarding the monitoring of the toad populations during the breeding season, as well as the monitoring requirements for some of the most important environmental parameters that also affect the toad conservation status.

5.1 Long term requirements

5.1.1 Population monitoring: frequency and period of monitoring

The monitoring programme carried out during three consecutive years (2004-2006) (present study) showed that there were important interannual variations in the number of egg strings laid at any given pond. Consequently, visits should be carried out each year, at least to a subset of the ponds (see below).

Based on the currently restricted distribution of natterjack toads in County Kerry (only 12 distinct sites), and the fact that many of these are likely to be isolated from the others, it is important to monitor each of the 12 sites. Within each site, some ponds are more used by the toads to breed and monitoring should primarily focus on these ponds (see Table 6.1). The condition at each site can then be assessed, based on the cumulative counts of egg strings at these selected ponds. Threshold values are given (see Table 6.1.). These correspond to approx 60% of the total number of egg strings recorded in 2004-2006 (see Table 3.1.), since future monitoring of toad populations is unlikely to cover the full breeding season. A comprehensive survey (all ponds) should also be carried out once every 5 years in order to provide a more accurate assessment of the toad status.

Table 5.1: Main ponds to be surveyed and minimum number of egg strings (cumulated over the breeding season) to be recorded, indicative of good status. Dry years correspond to years with low rainfall and/or low water levels during March-May. Wet years correspond to years with higher rainfall and/or higher water levels during March-May. The indicative number of egg strings corresponds to approx 60% of the total number of egg strings recorded in 2004-2006 (see Table 3.1.). An indicative number (minimum) of toadlets is provided, based on the 2004-2006 survey and on the ease at which toadlets can be observed at the ponds. The contribution of each pond (average over the period 2004-2006) is also given as percentage.

Breeding sites	**Ponds**	**Contribution**	**Number of egg strings**		**Toadlets**
			Dry year	**Wet year**	**Wet year**
Fermoyle	All 3 ponds	-	5	10	10s
Stradbally	Pond 1	35 %	100	200	High 1000s
	Pond 4	19 %	50	100	Low 1000s
Lough Gill	NW shore	92 %	15	30	High 100s
Maharees	Naparka	59 %	100	300	100s
	Pond 23	23 %	20	200	1000s
Tullaree	All 3 ponds	100 %	10	30	100s
Roscullen	Area 1 (pool)	85 %	50	300	1000s
L. Yganavan	Area 1	20 %	30	50	100s
	Area 4	28 %	30	70	100s
L. Nambrackdarrig	at stream	85 %	10	20	10s
Dooks	Pond 2	12 %	10	20	10s
	Pond 3	10 %	5	10	10s
	Pond 6 (field)	77 %	100	300	100s
Caherdaniel	Pond 1	34 %	20	80	Low 100s
	Pond 2	66 %	40	100	Low 100s

Based on the most recent survey of all toad populations (2004-2006, present study), it is possible to identify the period of the year when most of the egg strings are laid (see Figure 6.1) and when most of the toadlets are emerging from the ponds (see Figure 6.2). From Figure 6.1, it appears that during the period 2004-2006, spawning always started first at the southern sites and that the optimum time of the year to assess the number of egg strings produced at each pond is as shown in Table 6.2. In contrast, the pattern for the peak of emergence of toadlets is less clear, with no obvious differences between the populations at the North and the South of the species range. It appears that for most of the sites the toadlets will be at their maximum between mid June and mid July.

Alternatively, if it is not possible to carry out several site visits after mid June, it is useful to carry out additional visits in May and estimate the abundance of large tadpoles (with at least two back legs well developed, and/or with apparent front legs) as this provides a good indication of the maximum number of toadlets that will be produced approx. two weeks later.

Table 5.2: Period of the year for recording the number of egg strings and estimating the number of toadlets (minimum of three visits). In particular, we recommend surveys to be carried out on days following heavy rainfall, when toads are more likely to breed.

	Egg strings	**Toadlets**
Northern sites: *Fermoyle, Stradbally, Lough Gill, the Maharees, Tullaree*	Third week of April – Mid May (3 weeks)	Mid June – Mid July (5 weeks)
Southern sites: *Roscullen, Loughs Yganavan and Nambrackdarrig, Dooks, Glenbeigh and Caherdaniel*	April (4 weeks)	Mid June – Mid July (5 weeks)

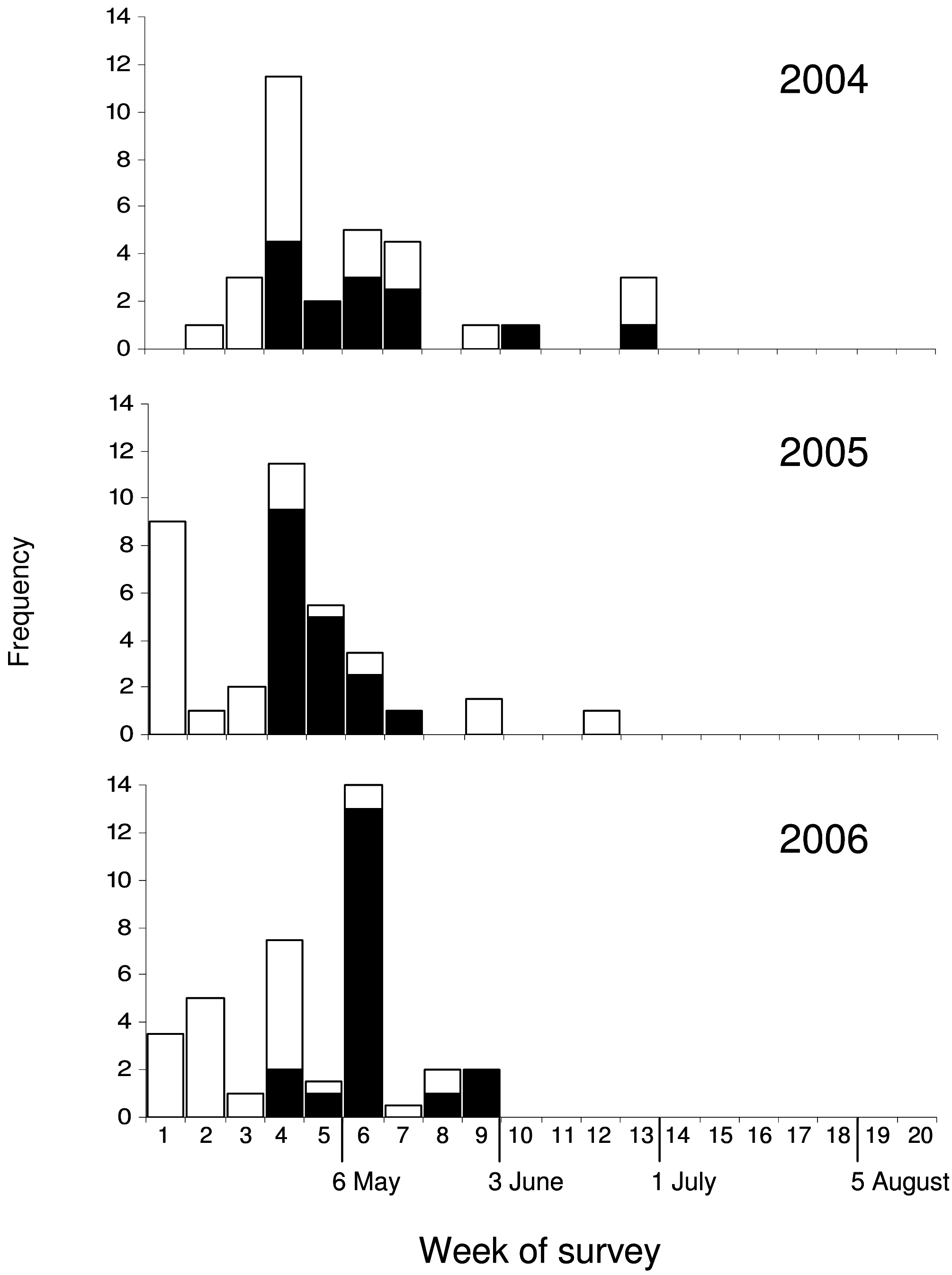


Figure 5.1: Period of the year where the maximum number of egg strings was recorded in single visits in 2004, 2005 and 2006 at each pond in the northern sites (black areas, comprising Fermoyle, Stradbally, Lough Gill, the Maharees and Tullaree) and in the southern sites (open areas, comprising Roscullen, Loughs Yganavan and Nambrackdarrig, Dooks, Glenbeigh and Caherdaniel). Time on the x-axis is expressed in weeks, with week 1 corresponding to the first week of April. The y-axis represents the number of ponds where the peak of egg strings was recorded during the corresponding week on the x-axis.

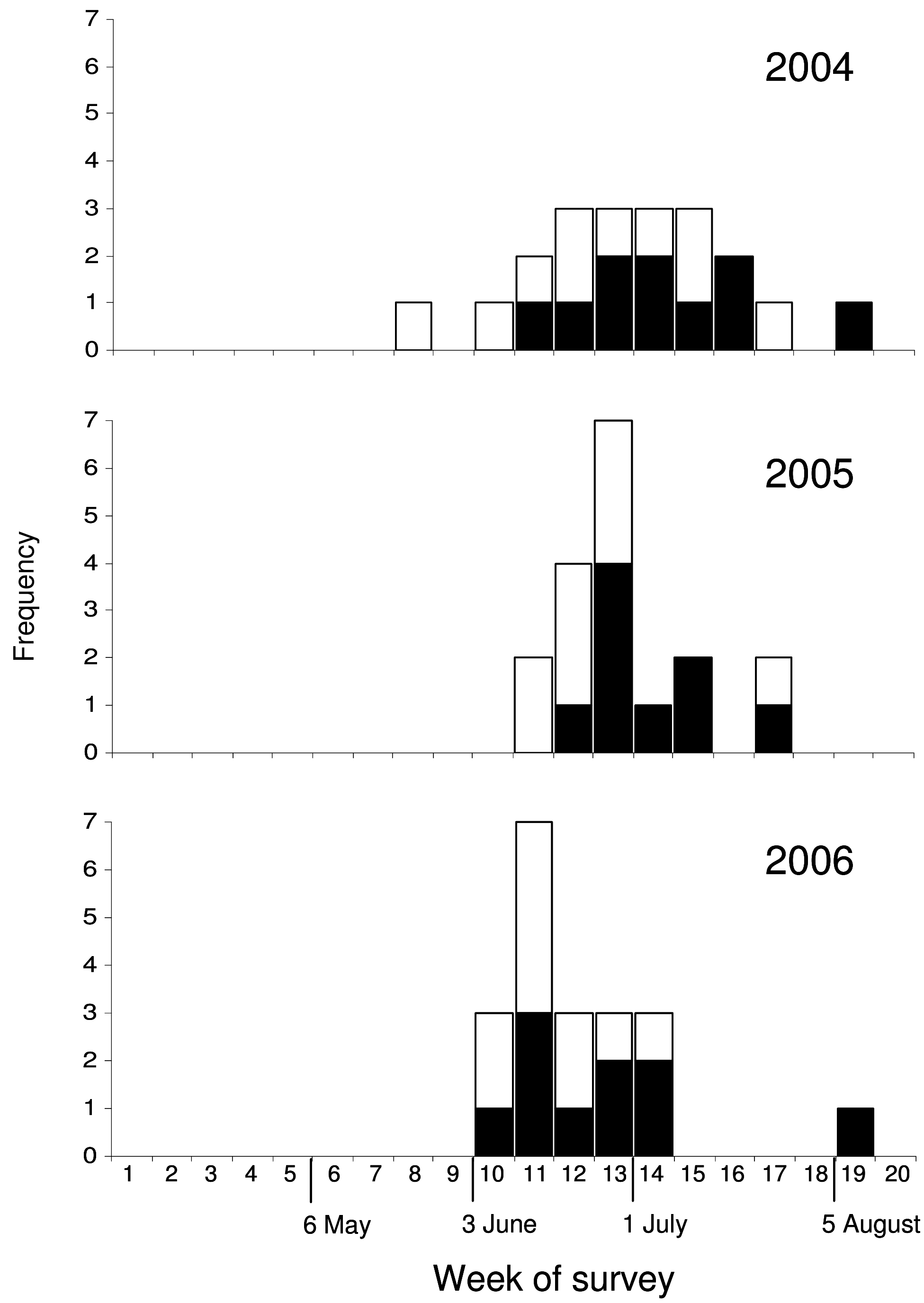


Figure 5.2: Period of the year where the maximum number of toadlets was recorded in single visits in 2004, 2005 and 2006 at each pond in the northern sites (black areas, comprising Fermoyle, Stradbally, Lough Gill, the Maharees and Tullaree) and in the southern sites (open areas, comprising Roscullen, Loughs Yganavan and Nambrackdarrig, Dooks, Glenbeigh and Caherdaniel). The time on the *x*-axis is expressed in weeks, with week 1 corresponding to the first week of April. The *y*-axis represents the number of ponds where the peak of toadlets was recorded during the corresponding week on the *x*-axis.

5.1.2 Population monitoring: methods

- **Egg strings:**

→ Walk along the entire perimeter of each pond and record the number of egg strings laid within the first 1.5m from the bank.

→ During cold periods, some egg strings might not have hatched when revisiting the pond after 8-10 days, so care must be taken not to double count egg strings.

→ The following ponds require use of waders, as many of the egg strings are also laid more than 2 metres away from the edge of the pond. At Caherdaniel, however, it is usually possible to see the egg strings from the shore, even when laid towards the middle of the pond. For the following ponds, transects should be carried out perpendicular to the shore in a jagged regular pattern when possible, and over 4-5 metres from the shore depending on the ponds.

1. Lough Gill**:** water levels can undergo marked fluctuations, and it is important to check the water at a distance of approximately 4 metres from the shore (for the breeding area close to the trench and sluice).
2. Maharees (Naparka): check the water approximately 5 meters from the shore, focusing on the Eastern half of the pond.
3. Maharees (Ponds 4, 23 and 25)
4. Roscullen
5. Lough Yganavan: some parts of the North East shore (Area 4) require surveying the water up to 4-5 meters from the shore.
6. Lough Nambrackdarrig
7. Dooks (Ponds 4, 5 and 6)
8. Glenbeigh (Field)

- **Toadlets:**
  - → Walk around the perimeter of the pond, up to a distance of 3-5 metres.
  - → Stop regularly to hand search toadlets hidden in the vegetation.
  - → Assess visually the density of toadlets per m^2 and the total area where the toadlets are present at this density. This provides a corresponding estimate of the total number of toadlets.

It is important to estimate the total number of toadlets as accurately as possible. A gross estimate of 10s, 100s or 1000s is not sufficient to distinguish differences among years and ponds.

- **Adults:**

We recommend carrying out adult night surveys at least once every three years at each site, anytime between 1st April and 15th May, and on a warm still night (to maximise the number of adults seen). During each night survey, the snout-vent-length of a sample of 35-45 individuals should be measured using a pair of callipers or a flat ruler, to the nearest millimetre. These data will allow the estimation of the mean size of breeding adults, and will also facilitate useful comparison of changes in the mean size of breeding adults through time (indicative of recruitment of young adults into the population).

5.1.3 Environmental parameters

The present study suggests that conductivity (and pH, which is correlated with conductivity) should not be too low. We therefore recommend measuring pH and conductivity at each pond using a pH/conductivity meter once every three years at each pond (in particular at Fermoyle, Lough Gill, Tullaree, Roscullen Island, and Nambrackdarrig) and also at each new potential breeding pond. On each site visit, pH and conductivity should be taken 3 times within the immediate vicinity of egg strings or tadpoles (readings to be taken within an area approximately $3m^2$).

5.1.4 Time required to survey natterjack toad populations for each of the three NPWS areas

FULL SURVEY (every 5 years):
Depending on the rainfall (and thus the amount of ponds (e.g. in the Maharees) and water surface area to survey (e.g. Roscullen)), we consider that the time required to survey each site is as follows (excluding night surveys):

- <u>Northern sites</u>: Fermoyle (3 ponds), Stradbally (7 ponds and drains), Lough Gill (1 area), the Maharees (1-7 ponds), Tullaree (5 ponds):

→ **Egg strings:** 3 surveys x 2 days per survey = **6 days**
→ **Toadlets:** 3 surveys x 2 days per survey = **6 days**

- <u>Southern sites</u>: Roscullen Island (2 areas and 4 new ponds), Inch (if possible) (2 areas), Lough Yganavan (4 areas), Lough Nambrackdarrig (1 area), Dooks (6 ponds) and Glenbeigh (2 ponds and 1-7 pools in the disused quarry):

→ **Egg strings:** 3 surveys x 3 days per survey = **9 days**
→ **Toadlets:** 3 surveys x 3 days per survey = **9 days**

- <u>Caherdaniel:</u>

→ **Egg strings:** 3 surveys x 1 hour = **3 hours**
→ **Toadlets:** : 3 surveys x 1 hour = **3 hours**

PARTIAL SURVEY (every year):
When only a subset of the ponds is surveyed (see Table 6.1.), the time required is as follows:
<u>Northern sites</u>: 3 surveys x 1 day = **3 days** for egg strings and **3 days** for toadlets
<u>Southern sites</u>: 3 surveys x 2 days = **6 days** for egg strings and **6 days** for toadlets
<u>Caherdaniel</u>: 3 surveys x 1 hour = **3 hours** for egg strings and **3 hours** for toadlets

5.2 Specific short term requirements

- Monitor any new breeding pools to assess the colonisation by natterjack toads
- During any site visit in areas adjacent to existing toad breeding sites, record (using GPS) potential other sites where breeding is occurring or where ponds could be created.

6. CONSERVATION AND MANAGEMENT RECOMMENDATION AT SPECIFIC SITES

6.1 Fermoyle

Although three egg strings were recorded in 2004 in Pond 3 at Fermoyle (see Figure 6.1), none were recorded either in 2005 or 2006. A few toadlets (10s) were observed in 2004. The spawning that occurred in 2004 permitted an estimate of the population size at this site (suggesting 9 breeding adults). The population growth analysis presented in section 3.5, shows that this site is likely to be in decline and the population vulnerable and close to extinction. Linking the Fermoyle site to the main natterjack population of the Maharees peninsula is highly desirable (Beebee 2002) by creating at least 4 ponds between Stradbally and Fermoyle. The site might also benefit from the re-introduction of eggs or tadpoles from some of the Strabdally ponds, especially if it is also the possible to create additional ponds westwards of Fermoyle, as previously suggested by Beebee (2002) and more recently by Shaw (2006).

The ponds at Fermoyle are relatively new. They were created to replace the loss of breeding waters that occurred in the early 1980s after drainage of the land and accompanying erosion. Although pH and conductivity seem adequate, these ponds are amongst the deepest of all the breeding ponds (see section 4.1.4). Shallow ponds are optimal for natterjack toad reproductive success. Although Ponds 1 and 3 present shallow margins, the water available for natterjack breeding is rather limited. In addition, no grazing took place at the site during the breeding season over the three years surveyed, allowing the phragmites reed bed to encroach substantially onto the pasture. Vegetation in the meadow was thus relatively high throughout the breeding seasons. High vegetation has been shown to be disadvantageous to natterjacks (Gent & Gibson 1998, Beebee & Denton 1996). We would recommend grazing by cattle at this site, especially as good fences have now been put in place around the deepest parts of each pond. We also recommend Pond 3 to be partly filled to better favour future reproduction.

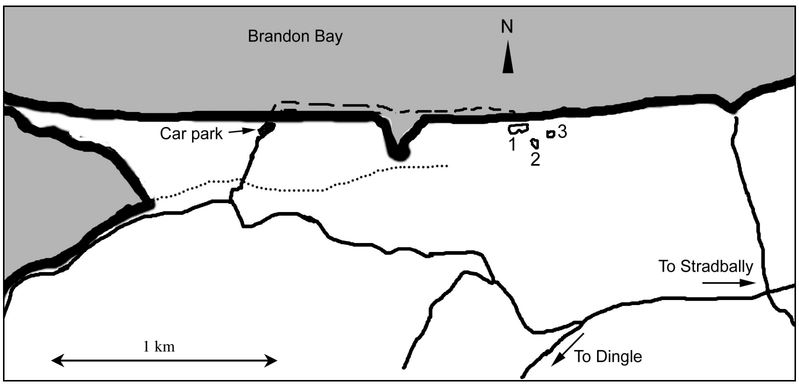


Figure 6.1. Natterjack toad breeding ponds at Fermoyle.
——— road, ... drain/river, – – – – – footpath.

6.2 Stradbally

Many toads were observed at this site during each of the three years of survey, although breeding was mainly concentrated to 3 ponds on the golf course (no breeding activity was observed along the shore of Lough Gill on the South eastern part of the golf course (Figure 6.2)). The egg strings recorded in 2006 permitted an estimate of the population at just over 3,000 adults. Thus Stradbally represents the 2nd largest population of the Co. Kerry region (see section 3.1). Breeding also appeared to be relatively successful (high survival rates), however fecundity was very low compared to the average at other sites. Nevertheless, the population here seems to be at equilibrium, based on the estimated growth rate (see section 3.5).

Ponds 1, 2 and 4 seem to be adequate and allow for successful breeding. In contrast, the other ponds (Ponds 3, 5, 6 and 8) attract less natterjacks and survival of eggs and tadpoles is lower at these ponds. This is possibly due to the presence of abundant aquatic vegetation and steeper banks. Survival in these ponds may also be limited by predation by insect larvae. In particular, water beetle larvae (*Dytiscus* spp) and dragonfly larvae (*Sympetrum* spp) were often observed at ponds 5, 6 and 8. Filling these ponds partially and clearing the vegetation may allow them to be more successful for natterjack reproduction. Nevertheless, the management regime at this site (e.g. restricts public access to the ponds) should be continued.

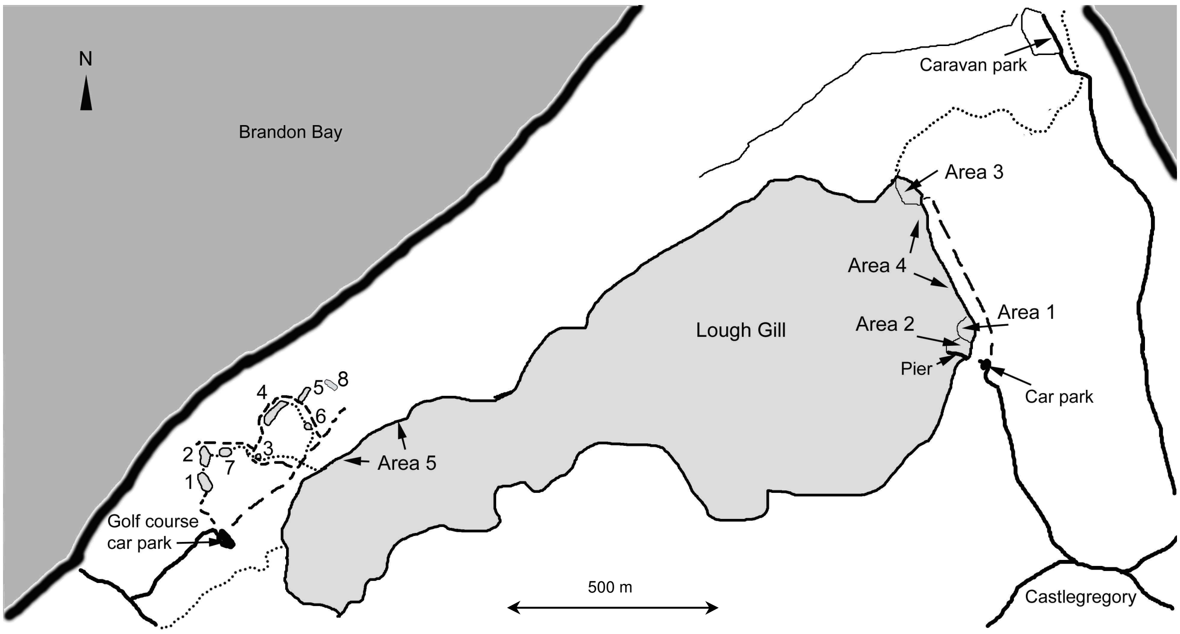


Figure 6.2. Natterjack toad breeding ponds at Stradbally golf course and Lough Gill. ———— road, ········ drain/river, ———— footpath.

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6.3 Lough Gill

Breeding at this site seems to be successful. Survival appeared to be relatively high compared to the other sites. Although the sluice was repaired in 2006, fluctuations of the water level made it difficult to accurately survey the spawning activity over the three years of study. Egg strings were probably missed each year as the water level increased and made the spawns inaccessible. In 2005, water level fluctuation caused mortality of egg strings, which had been laid in the shallowest parts of the bay (NW shore). The water levels receded before many eggs developed into tadpoles. Despite the high mortality of many egg strings, many toadlets (high hundreds) were observed each year. Therefore the population was probably underestimated and the survival rates over estimated. Based on these estimations, the population growth rate was estimated to be greater than 1, indicating that the population is growing. It is difficult to validate the results of the modelling work based on the uncertainties over our egg string counts and survival rate estimations. Overall the current conditions at this site appear favourable. It is however essential to maintain a sufficient level of grazing by cattle along the Northwest shore of the Lough. The absence of grazing would otherwise lead to abundant growth of reeds and Iris beds and reduce the suitability of the breeding habitat.

6.4 The Maharees

The Maharees population appeared to be the largest in Co. Kerry during 2005 and 2006, supporting close to 3,500 individuals in 2006 (see section 3.1). Breeding activity was very important at this site, with the Maharees female natterjacks being the most fecund of all populations (see section 3.3). Usually spawning was observed as soon as ponds formed after rainfall, some ponds were nevertheless ephemeral and dried before eggs developed into tadpoles or toadlets. Consequently survival at this site was rather low. Historically a large number of ponds formed in the Maharees, although now this tends not to be the case. Beebee (2002) reported that up to 25 ponds could form in the dune system (see Figure 6.3). Over the three years of survey, no more than 9 ponds formed and were used for breeding. We suspect that a drop in the water table (due to possible changes in water resource utilisation rather than recent changes in climate (Aubry & Emmerson, 2005) could be the cause of such an alteration.

In addition, it seems that there has been an increase in the number of cattle and other animals (horses) on the dunes at this site. Intake of water by these animals is substantial. On average a cow can drink up to 40 litres of water per day (Irwin, 1992). Becart *et al.* . (2006) estimated the daily water intake of the cattle in the Maharees to be over 8,600 litres. It is apparent that such a large intake contributes to the impact on natterjack egg and tadpole survival by speeding the rate at which a pond will dry out. Intense trampling by the cattle in and around the ponds may also contribute to increased mortality of eggs and tadpoles. There is a fine balance to achieve between the presence and over stocking of cattle at natterjack breeding sites.

The growth rate estimated for the population in the Maharees is less than 1. Because fewer ponds have formed over the past decade and recruitment has been limited by rapid pond dessication (over the 2004-2006 study period), we consider the Maharees population to be declining. The conditions at this site would be optimal if the ponds could hold water for a sufficient period of time to allow recruitment. It would be informative to study the age distribution of the individuals in this population to better evaluate its health and sustainability. Older females tend to lay longer egg strings (Beebee, 1979, 2002), and the average number of eggs per string at this site is higher than the Co. Kerry average. We therefore hypothesise that the Maharees population is composed of older individuals, which might also indicate a senescent population and lack of recruitment. Some of the Maharees dune slacks have areas of localised disturbance due to intense cattle poaching. In these areas deepening of the locally disturbed areas might contribute to an increased hydroperiod of the ponds (and hence successful natterjack breeding), although it should also be noted that the dune slacks in the Maharees are characteristic of "Humid Dune Slacks", a habitat listed in Annex I of the EU Habitats Directive.

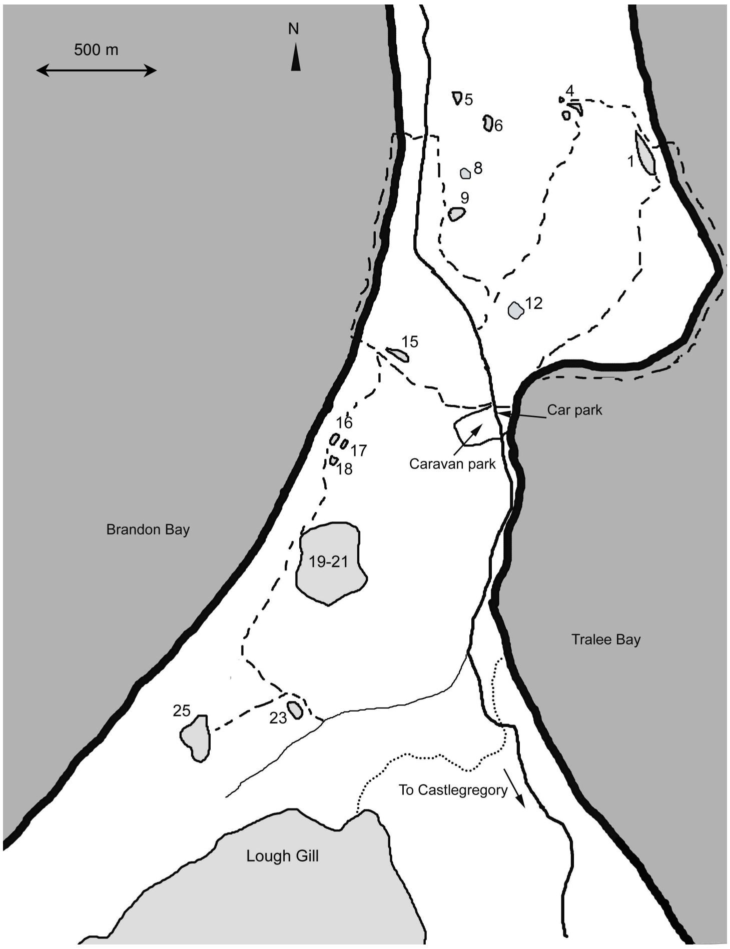

Figure 6.3. Natterjack toad breeding ponds in the Maharees.
——— road, ········· drain/river, – – – – – – footpath.

6.5 Tullaree

The population at Tullaree is rather small. We estimate that approximately 160 adult toads breed at this site (section 3.1). The population is among the smallest in Co. Kerry. However, our estimates of survival suggest that the site is one of the most successful (see sections 3.4 and 3.5), with a growing adult population. Conditions at this site are therefore favourable. The ponds are shallow and warm, which speeds egg development, through to tadpoles and toadlets. Conductivity is amongst the highest recorded. At the close of 2006, the aquatic vegetation was particularly abundant in all ponds, (despite the previous attempts to clear vegetation in 1999), limiting the surface available for breeding. A recent project funded by the Heritage Council within their Biodiversity Fund 2006 scheme resulted in the improvement of 2 of the 3 existing pools (pools 1 and 2) and the creation of 2 additional pools in very close proximity (Shaw 2006). Also in 2006, NPWS supervised the erection of fences in order to reinstate grazing by horses, which will limit vegetation growth in and around the ponds.

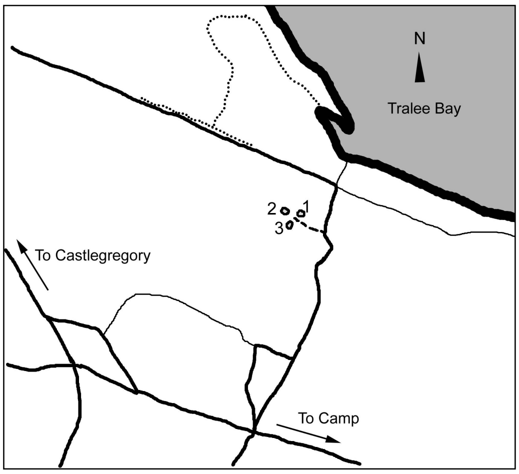

Figure 6.4. Natterjack toad breeding ponds at Tularee.
——— road, ········ drain/river, – – – – – footpath.

6.6 Roscullen Island

The 2004 to 2006 survey suggests that Roscullen Island is the most successful natterjack breeding population. In 2006, the site was estimated to support approximately 2,700 breeding adults, representing the third largest Irish population (see section 3.1). In each year of study, spawning always occurred first at Roscullen Island. Fertility was estimated to be close to the average for all the Co. Kerry populations (despite the low sample size) (see section 3.3.1). The growth rate estimated in section 3.5 indicates that the population is expanding. The area of available breeding habitat is large, and there is little evidence for density dependence at the site. Sufficient water is maintained on the site to allow for successful breeding and development of tadpoles, until emergence of toadlets. Conductivity is relatively high, but still within the range tolerated by the species. The site is moderately grazed, typically during the second half of the breeding season, therefore limiting vegetation growth. The current conditions at Roscullen Island are therefore very favourable. The site would be particularly vulnerable to improved drainage (ditch improvement) and would suffer if grazing was reduced.

An area of breeding water (North West of A2, see Fig. 6.5) discovered at the end of 2004 was largely destroyed in 2005 following the deepening of a large drain, whilst adult natterjacks were still present in the area in 2006 no breeding activity was recorded. In August 2006, four new ponds were created North West of the main breeding area to increase the area available for breeding (Shaw 2006). Even though Roscullen Island supports a large population, the site is isolated. It would be desirable to link Roscullen Island with Inch to the West and with other possible breeding sites to the East around Castlemaine Harbour (South Dingle Peninsula).

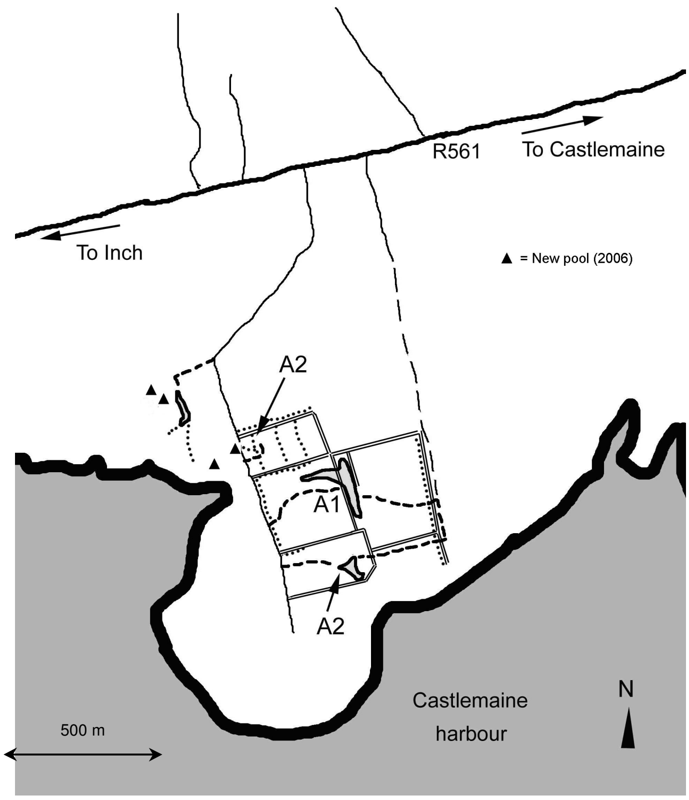

Figure 6.5 Natterjack toad breeding areas at Roscullen Island.
——— road, ... drain/river, - - - - - footpath, ═══ fence

6.7 Inch

No regular visits were carried out at this site over the three years of study. During two visits in 2005 and 2006, egg strings were observed in a dry ditch, on the eastern side of the sand dunes as well as hundreds of tadpoles in a pool further South. We support Beebee's (2002) recommendations to improve the freshwater habitat and deepen some slacks so that water can be held throughout the breeding season and allow recruitment. Although, the population at this site is poorly documented, we suspect that there is an imminent threat of local extinction. We believe the number of adult toads to be small (low 100s) and no recruitment seems to be taking place. Historical evidence suggests that the site once hosted a thriving natterjack population (Beebee 2002). Local conditions are suitable for natterjacks, but would require active and sympathetic management as in the Maharees. The limiting factor on the Inch site seems to be the availability of sufficient standing water in which the toads can breed. Linking the populations of Inch and Roscullen Island, through the creation of at least 10 ponds along the northern edge of Castlemaine harbour is also highly desirable (Beebee 2002). Increasing population connectivity would reduce the risk of local population inbreeding and extinction.

6.8 Lough Yganavan

The population at Lough Yganavan was estimated at approximately 1,300 breeding adults (see section 3.1), corresponding to a relatively large population. However, we would expect a greater population size, considering the large area of habitat available. Breeding has been relatively consistent over the three years of survey but fertility is rather low (below 2,000 eggs per string) (see section 3.3). The growth rate estimated in section 3.5 is close to 1, indicating that the population at this site is stable. The conditions in some areas (Areas 3 and 4 (see Figure 6.6)) are adequate, with shallow warm water facilitating successful breeding. However, in some other parts (Areas 1 and 2 (see Figure 6.6)), the water is deeper and thus colder early in the season. Egg strings are vulnerable to *Saprolegnia* in such conditions (Banks & Beebee 1988), especially in the presence of fish which are a potential vector of this disease. In 2005, all the egg production in Area 1 was lost following infection by a fungus. In addition, egg strings were often found supported by a dense mat of vegetation (*Hypericum elodes*) in these areas and they were thus more susceptible to predation. Even though many tadpoles were observed in Area 2 in 2006, no toadlets were found. Toadlets could not be seen easily among the high vegetation around Area 1. We suspect the numbers to be higher since many of the tadpoles were observed at a late developmental stage.

Only two thirds of the lough were surveyed from 2004 to 2006. We were not able to check for any breeding activity in the remaining part of the Lough (i.e. South and South West shores). However, we suspect breeding also takes place at the western part of the Lough where toads have been heard calling. Further investigation should be carried out, especially as some toads have previously been recorded on the southern shore in the 1980s (Gibbons 1981).

Many housing developments occurred near this site during the three-year study. It is important that such developments are controlled to avoid a negative impact on the population here and any further loss of habitat. Some parts of the shore would benefit from vegetation clearance, in particular along the northern parts of the Lough where dry-stone-walls, which used to act as hibernation sites, have now been overgrown by gorse, grass and moss (Shaw 2006).

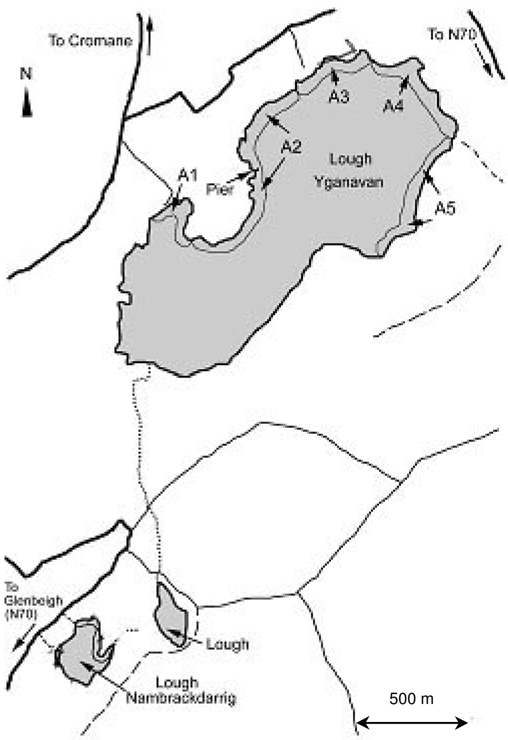

Figure 6.6. Natterjack toad breeding areas at Lough Yganavan and Lough Nambrackdarrig. ——— road; ········· drain/river; – – – – – – footpath.

6.9 Lough Nambrackdarrig

This site supports the second smallest population in Co. Kerry. We estimated the number of breeding adults to be 50 in 2006 (see section3.1). Breeding success was very low, with less than 0.1% egg to toadlet survival throughout the three years of survey (see section 3.4.2). We estimated that the population growth rate was significantly less than 1 (see section 3.5), indicating that the population is in decline. Anecdotally, male natterjack calling has declined in recent years at Nambrackdarrig. Conditions at this site are atypical for natterjacks; the lough is bounded by bog, and the pH and conductivity are very low. The water is relatively deep and egg strings were often found supported by vegetation (immerged *Sphagnum* sp and vegetation structure dominated by *Hypericum elodes* and *Menyanthes trifoliata*) and thus close to the water surface. The assessment of the number of toadlets was made difficult by the high vegetation (*Juncus* sp) along the border of the lough. The same vegetation did seem to provide a refuge for tadpoles. The environment remained damp throughout the breeding season, and toadlets were found in tens among *Juncus* sp. and *Sphagnum*. We may therefore have underestimated survival. The small population size at Nambrackdarrig means that the population's status is not favourable. Conservation, maintenance and clearing of the dry-stone-walls in the vicinity of Lough Nambrackdarrig could provide more over-wintering sites for the natterjacks and we recommend that this be undertaken with some urgency.

6.10 Dooks

Breeding activity was variable over the study period (2004-2006), 45 egg strings were recorded in 2004, 568 in 2005, 209 in 2006 (see Table 3.1 in section 3.1). It is thus difficult to accurately estimate the population size. Estimates vary from low 100s to low 1,000s of breeding adults. Fertility was close to the average estimated for all the Co. Kerry populations (see section 3.3), however, egg to tadpole and egg to toadlet survival rates were very low. The estimated population growth rate (see section 3.5) suggests that the population may be in decline.

Most of the breeding activity observed at Dooks took place at Pond 6 (in a field located 0.5 km away from the golf course), whilst 5 ponds are present on and immediately adjacent to the golf course. Spawning at Pond 6 accounted for 88% of the total spawning recorded at this site in 2005, and 65% of the total spawning recorded in 2006 (see section 3.1). The ponds on the golf course (Ponds 1 to 3) do not support a large breeding population, which is surprising. A possible explanation is that the natterjack population is small on this part of the site due to other limiting factors acting via the terrestrial habitat.

Pond 1 (on the golf course) is unsuitable for natterjacks; breeding activity is particularly low, and tadpole predators were often seen in the water. This pond is deep and presents a high biomass of algae and macroflora, likely due to high nutrient loads. In addition, the presence of bacteria (noticed from the red aspect of the water) suggests anoxic conditions. Ponds 2 and 3 are shallow and hold water until the end of the breeding season (except in dry years such as 2004). These two ponds do not hold abundant vegetation, and because they dry each year predation risk by insect larvae is limited. In contrast, Ponds 4 and 5, on the adjacent commonage provide less favourable conditions for breeding. Pond 5 has a low pH compared to the other ponds (see Appendix 3). Cattle and sheep regularly graze the vegetation around Ponds 4 and 5.

Pond 6 (field) is large, but shallow, with deep mud in places. The vegetation within (Charaphytes) reduces the effective water depth (distance between the egg strings and water surface area), making the whole water surface available for breeding by toads (egg strings were observed across the entire surface of the pond). The southern borders are also very shallow, and high concentrations of tadpoles were observed all along this shoreline (several 10,000s). Cattle graze the site during the first half of the breeding season, limiting vegetation height and opening the ponds margins through trampling. The high vegetation (irises) and soft substrate, along with many hummocks due to trampling, provided refuges for toads, and made it difficult to observe toadlets at this pond.

We would recommend the vegetation to be cleared from Ponds 1, 4 and 5 and Pond 4 to be deepened to hold water longer. These actions would probably aid recruitment and maintain the population present on the golf course. The management regime employed at Pond 6 should be maintained (i.e. moderate level of grazing). We would also recommend the creation of additional ponds between Pond 6 and the golf course (on the adjacent commonage) to increase the connectivity of these two subpopulations.

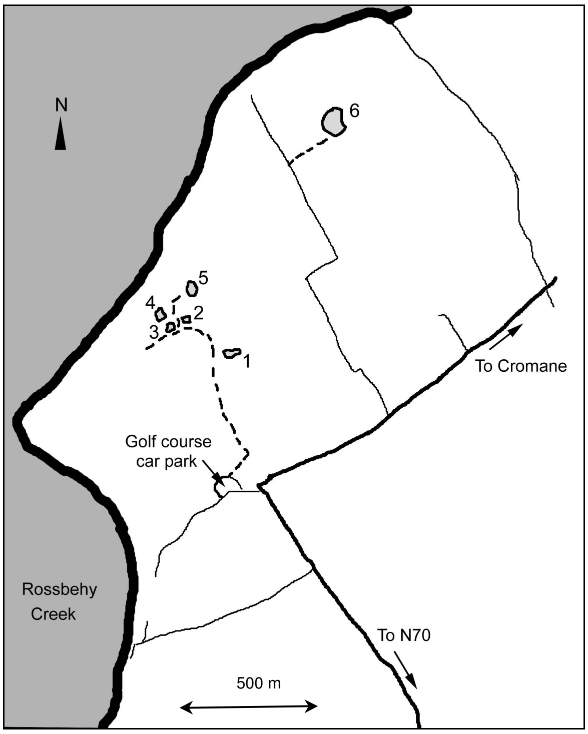

Figure 6.7. Natterjack toad breeding ponds at Dooks.
——— road, - - - - - footpath.

6.11 Glenbeigh

The adult population at Glenbeigh is one of the smallest in Co. Kerry and arguably one of the most vulnerable. Over the period of the present study considerable development has taken place in the town of Glenbeigh and natterjack breeding habitat is at risk. We estimate that there are between 160 and 200 breeding adults at Glenbeigh (see section 3.1). Breeding success is low and the population growth rate estimated in section 3.5 indicates a likely decline of the population. This site is composed of three spatially discrete breeding areas: a disused quarry, a field (commonage), and an area of marsh.

6.11.1 Marsh

Much of the marsh breeding area fronting the sea at Glenbeigh has disappeared. This habitat has been lost over the past decade due to a faulty sluice (see Beebee 2002). The broken sluice permitted the regular intrusion of seawater to the freshwater marsh. These ponds located just behind the sea wall had reverted to saltmarsh and were not suitable for natterjacks.

In 2005, a brackish pool was found on privately owned land (to the west of the sluice), and some breeding took place there in both 2005 and 2006. Natterjacks are still present at the site and the newly discovered pool has contributed toadlets to the local population. However, the pond is situated on privately owned land where recent marsh reclamation has taken place. In 2006, two pools were excavated within the old marsh, and the sluice gate was repaired. Volunteers from UCC and the Herpetological Conservation Trust cleared encroaching scrub (mostly gorse and brambles) from a small area on the eastern side of the sluice (Shaw 2006).

6.11.2 Disused quarry

The disused quarry (see Figure 6.8), comprised approx. 7 ephemeral pools. Tens of egg strings were observed each year at the beginning of the season (see section 3.1). The ponds were very shallow and tadpoles at this site developed quickly. However, the ephemeral ponds at the quarry commonly dried before the emergence of tadpoles (in 2005, all the pools were dry by the 5th of May). Additional ponds were discovered on the site amongst trees and bushes in 2005, but these ponds were not used for breeding by natterjacks.

During the 2006 breeding season, a large area of the quarry was cleared of vegetation (mostly gorse). The scrub clearance was achieved using heavy machinery, 4 ephemeral pools were destroyed and it is likely that significant mortality among adults occurred. However, in the process, a number of new shallow scrapes were formed and held water well into the breeding season. Breeding took place successfully and 16 egg strings were recorded, tens of thousands of tadpoles observed and low hundreds of toadlets. The conditions at this site have thus become more suitable for breeding.

The pH recorded at the quarry site was also amongst the lowest of all sites; the vegetation may have contributed to the acidic conditions on the site (Gorse, Heather and *Shagnum* sp) (see Appendix 3 and section 4.1). Although the local disturbance has had a beneficial effect, the purpose of the clearance remains unknown and may have represented land clearance for development. This particular case illustrates how sensitive the toad's breeding habitat is to future development.

6.11.3 Commonage

Finally, the commonage breeding site at Glenbeigh showed considerable variability in breeding activity over the three years of study. In 2004, 28 egg strings were recorded, 51 in 2005, and 14 in 2006. The pond is situated in a boggy field, and is filled with vegetation (mainly *Hypericum elodes* and *Shpagnum* sp). The pH is amongst the lowest of all sites (see Appendix 3). The pond is rather deep but the vegetation allows egg strings to be laid close to the surface. Many tadpoles were observed but very few developed into toadlets. Predation may be an important component of mortality at this site. Grazing did take place at the pond in 2004, fewer cattle were present in 2005, and none were present in 2006. In 2005, a hard-core road approximately 4 metres by 100 metres was constructed to enable the access to an adjacent field. It is important to avoid further loss of habitat, especially as the population at Glenbeigh currently relies almost entirely on this breeding site. It is highly desirable to reinstate low intensity grazing by cattle in and around the pond.

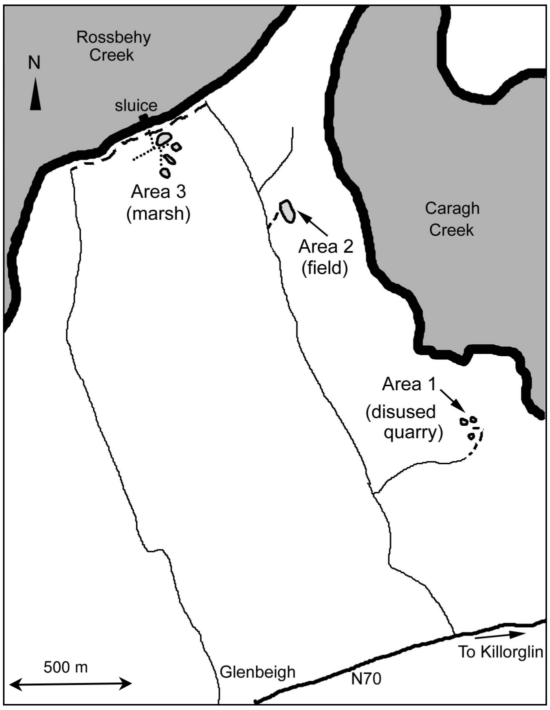

Figure 6.8. Natterjack toad breeding areas at Glenbeigh.
——— road, ······ drain/river, – – – – – – footpath.

6.12 Caherdaniel

The population at Caherdaniel was re-introduced in the early 1990s. Whilst breeding activity was relatively low in 2004 (98 egg strings recorded), there was an increase in 2005 and 2006 (333 and 313 respectively (see section 3.1). We estimate the population to be comprised of approximately 1,000 breeding adults (a relatively large population). However, breeding success was low (egg to toadlet survival <0.1% (see section 3.4)), and the estimated population growth rate (see section 3.5) indicates that the population is likely to be in decline. Females at this site tend to lay longer egg strings (n=2790) compared to the average of the Co. Kerry populations (n=2195) (see section 3.3). As in the Maharees, this may indicate an older population (since older females tend to lay longer egg strings (Beebee 1979)).

Over the three years of survey, no or little recruitment took place (i.e. a few toadlets were observed). This is certainly due to a high rate of predation. Many large predators were observed in the ponds, including leeches, greater water beetle larvae, and dragonfly larvae. One of the ponds was partly filled at the end of the 2006 breeding season thus making it shallower (Shaw 2006). It will be important to monitor this pond during the next breeding season in order to assess the breeding success under the new conditions. Another pool was also created between the two existing ponds to increase the chance of breeding success. We would however recommend other ponds to be created in a different area to allow migration and genetic flux to take place. The population in Caherdaniel is isolated from the main populations on the Iveragh and Dingle Peninsula, and inbreeding is likely to take place. Furthermore, the creation of a new site nearby would help restore the historical status of natterjack in the area, which used to breed in Coomakista further to the west.

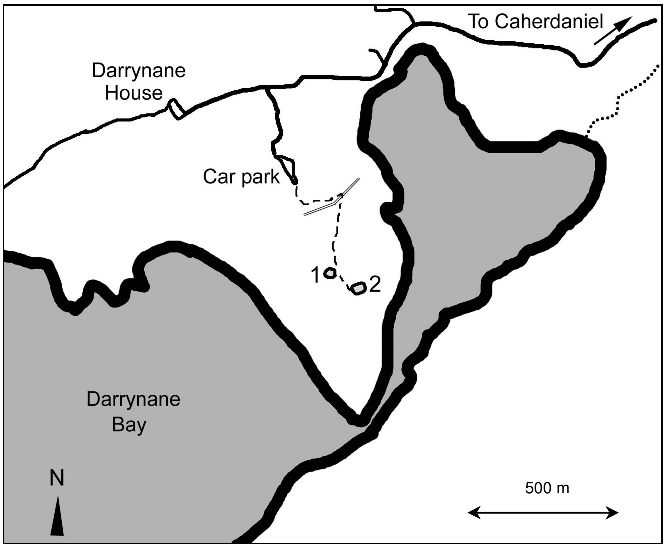

Figure 6.9. Natterjack toad breeding ponds at Caherdaniel.
——— road, ······· drain/river, – – – – – – footpath, ═══ fence.

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Appendix 1

Table A: Categorical environmental variables for each pond over the period 2004-2006

Site	Pond	Habitat	Activity			Pond permanency		
		2004-2006	2004	2005	2006	2004	2005	2006
Fermoyle	Pond 1	4	3	3	3	2	2	1
	Pond 2	4	3	3	3	1	1	2
	Pond 3	4	3	3	3	1	1	1
Stradbally	Pond 1	1	2	2	2	1	1	1
	Pond 2	1	2	2	2	1	1	1
	Pond 3	1	2	2	2	1	1	1
	Pond 4	1	2	2	2	2	2	2
	Pond 5	1	2	2	2	1	1	1
	Pond 6	1	2	2	2	1	1	1
	Drains	1	2	2	2	3	3	3
	Pond 8				2			1
Lough Gill	N-W shore	4	1	1	1	2	2	2
	Other areas	4	3	3	3	2	2	2
Maharees	Pond 1	1	1	1	1	3	3	2
	Pond 4	1	1	1	1	3	3	3
	Pond 8	1	1	1	1	3	3	3
	Pond 9	1	1	1	1	3	3	3
	Pond 12	1	1	1	1	3	3	3
	Pond 15	1	1	1	1	3	3	3
	Pond 16	1	1	1	1	3	3	3
	Pond 23	1	1	1	1	3	3	3
	Pond 25	1	1	1	1	3	3	3
Tullaree	Pond 1	5	3	3	3	2	2	2
	Pond 2	5	3	3	3	2	2	2
	Pond 3	5	3	3	3	1	1	1
Roscullen	Area 1	5	1	1	1	3	3	2
	Area 2	5	1	1	1	3	3	3
Yganavan	Area 1	2	3	3	3	1	1	1
	Area 2	2	3	3	3	1	1	1
	Area 3	2	1	1	1	1	1	1
	Area 4	2	1	1	1	2	2	2
	Area 5	3	3	3	3	2	2	2
Nambrackdarrig		3	3	3	3	1	1	1
Dooks	Pond 1	2	2	2	2	1	1	1
	Pond 2	2	2	2	2	3	2	2
	Pond 3	2	2	2	2	3	2	2
	Pond 4	2	1	1	1	3	3	3
	Pond 5	2	1	1	1	3	3	3
	Pond 6	4	1	1	1	1	1	1
Glenbeigh	Quarry	2	3	3	3	3	3	3
	Field	3	3	3	3	3	3	3
	Marsh	5	3	3	3			1
Caherdaniel	Pond 1	1	3	3	3	1	1	1
	Pond 2	1	3	3	3	1	1	1

- **Habitat types:** 1) Early sand dune, 2) Scrub (includes sand dunes in late stages), 3) Bog and Heathland, 4) Improved grassland and 5) Marsh.
- **Activity types:** 1) Farming activity, 2) Golf course and 3) Discontinuous land use (i.e. low degree of human intervention).
- **Pond permanency:** 1) permanent, 2) semi-permanent (reduced by at least half its size of April before the end of the breeding season (31st July)) and 3) ephemeral (dried out before the end of the breeding season).

Appendix 2

Table B: Results of the Tukey's test performed on the fertility of each site. The table presents the degrees of significance and the P-values for each pair-wise comparisons. * refers to P<0.05, ** refers to P<0.01, ***refers to P<0.001

	Stradbally	**L.Gill**	**Maharees**	**Roscullen**	**L.Yganavan**	**Dooks**	**Caherdaniel**
Stradbally		NS	***	NS	NS	***	***
L.Gill	0.655		NS	NS	NS	NS	NS
Maharees	<0.001	0.608		NS	***	NS	NS
Roscullen	0.257	0.999	0.933		NS	NS	NS
L.Yganavan	0.198	0.980	<0.001	0.757		NS	***
Dooks	0.001	0.998	0.377	1	0.126		NS
Caherdaniel	<0.001	0.922	0.608	0.000	<0.001	0.956	

Appendix 3

Table C: Environmentnal parameters measured in 2004 for each pond at each site. "–" is employed when no measure was taken. Conductivity is expressed in µS.cm-1, water temperature in °C, water depth in cm and salinity in ppt.

		pH	± SE	Cd	± SE	T°C	± SE	WD	± SE	Sal	± SE
Dingle Peninsula-North											
Fermoyle	Pond 1	8.4	± 0.1	486	± 26	17.8	± 2.0	14.9	± 2.0	-	-
	Pond 2	8.4	± 0.1	549	± 43	18.3	± 1.7	14.1	± 0.5	-	-
	Pond 3	8.2	± 0.1	640	± 7	20.0	± 1.4	14.0	± 0.9	-	-
mean		**8.3**	**± 0.1**	**558**	**± 45**	**18.7**	**± 0.6**	**14.3**	**± 0.3**	-	-
Stradbally	Pond 1	8.9	± 0.1	379	± 15	18.2	± 1.1	12.0	± 1.8	-	-
	Pond 2	9.1	± 0.2	395	± 9	18.7	± 1.2	12.8	± 1.7	-	-
	Pond 3	8.2	± 0.1	529	± 22	17.1	± 1.2	22.6	± 2.8	0.0	± 0.0
	Pond 4	8.7	± 0.2	434	± 20	18.8	± 1.1	9.6	± 1.4	-	-
	Pond 5	8.5	± 0.1	525	± 16	17.1	± 1.1	20.3	± 2.4	-	-
	Pond 6	7.8	± 0.1	600	± 13	17.6	± 1.1	15.7	± 2.4	-	-
	Pond 7	7.9	± 0.4	598	± 45	15.9	± 0.6	18.9	± 0.1	0.1	± 0.0
	Pond 8	-	-	-	-	-	-	-	-	-	-
mean		**8.3**	**± 0.4**	**494**	**± 35**	**17.6**	**± 0.4**	**± 16.0**	**± 1.8**	**0.1**	**± 0.1**
Lough Gill	N-W sh	7.8	± 0.3	4724	± 1988	18.6	± 1.4	9.6	± 1.1	2.7	± 1.3
	Other	8.2	± 0.3	3441	± 703	18.2	± 1.3	10.4	± 1.3	2.0	± 0.4
mean		**7.9**	**± 0.4**	**4082**	**± 641**	**18.4**	**± 0.2**	**10.0**	**± 0.4**	**2.3**	**± 0.4**
Maharees	Pond 1	8.1	± 0.3	764	± 60	18.9	± 1.5	9.4	± 0.9	0.1	± 0.1
	Pond 4	8.0	± 0.3	689	± 59	18.1	± 2.6	9.6	-	-	-
	Pond 8	-	-	-	-	-	-	-	-	-	-
	Pond 9	-	-	-	-	-	-	-	-	-	-
	Pond 12	-	-	-	-	-	-	-	-	-	-
	Pond 15	8.7	± 0.3	667	± 118	18.0	± 2.9	7.3	-	-	-
	Pond 16	-	-	-	-	-	-	-	-	-	-
	Pond 23	8.2	± 0.4	587	± 126	16.6	± 0.6	9.0	± 1.8	-	-
	Pond 25	8.7	± 0.2	563	± 102	15.3	± 0.6	10.0	-	-	-
mean		**8.2**	**± 0.5**	**654**	**± 36**	**17.4**	**± 0.6**	**9.1**	**± 0.5**	-	-
Tullaree	Pond 1	7.1	± 0.1	582	± 38	16.2	± 1.4	15.8	± 1.1	0.1	± 0.0
	Pond 2	7.2	± 0.1	1976	± 573	17.1	± 1.2	14.9	± 0.8	1.1	± 0.4
	Pond 3	7.3	± 0.1	770	± 74	16.9	± 1.5	20.5	± 1.8	0.2	± 0.1
mean		**7.2**	**± 0.1**	**1109**	**± 437**	**16.7**	**± 0.3**	**17.1**	**± 1.7**	**0.5**	**± 0.3**

Table C: Continued

		pH	± SE	Cd	± SE	T°C	± SE	WD	± SE	Sal	± SE
Dingle Peninsula-South											
Roscullen Island	Area 1	6.1	± 0.3	2087	± 420	18.2	± 1.0	10.2	± 1.0	-	-
	Area 2	6.2	± 0.1	802	± 169	14.3	± 1.0	11.2	± 1.0	-	-
mean		**6.2**	**± 1.1**	**1444**	**± 642**	**16.2**	**± 1.9**	**10.69**	**± 0.5**	-	-
Iveragh Peninsula-North											
L.Yganavan	Zone1	6.6	± 0.2	149	± 5	18.8	± 1.0	8.3	± 1.0	-	-
	Zone2	6.6	± 0.1	126	± 0	15.4	± 1.0	12.7	± 1.0	-	-
	Zone3	6.9	± 0.1	156	± 4	22.2	± 1.0	6.7	± 1.0	-	-
	Zone4	6.5	± 0.5	168	± 8	24.4	± 1.0	6.4	± 1.0	-	-
	Zone5	6.2	± 0.3	150	± 7	19.0	± 1.4	4.6	± 0.3	-	-
mean		**6.2**	**± 0.3**	**164**	**± 7**	**19.9**	**± 1.5**	**7.7**	**± 1.4**	-	-
L.Nambrackdarrig		**5.1**	**± 0.2**	**123**	**± 2**	**17.4**	**± 0.7**	**20.4**	**± 1.2**	**-**	**-**
Dooks	Pond 1	7.9	± 0.3	276	± 9	17.3	± 2.0	8.0	± 1.0	-	-
	Pond 2	6.8	± 0.2	179	± 11	16.6	± 2.0	11.1	± 1.0	-	-
	Pond 3	6.6	± 0.1	175	± 12	15.1	± 1.0	15.6	± 3.0	-	-
	Pond 4	6.3	± 0.3	186	± 32	15.5	± 3.0	10.5	± 2.0	-	-
	Pond 5	6.0	± 0.4	201	± 19	16.1	± 2.5	17.4	± 3.0	-	-
	Pond 6	8.4	± 0.6	319	± 12	19.5	± 2.0	15.9	± 2.0	-	-
mean		**7.0**	**± 0.3**	**223**	**± 25**	**16.7**	**± 0.7**	**13.1**	**± 1.5**	-	-
Glenbeigh	Quarry	4.9	± 0.2	124	± 24	16.5	± 3.0	5.2	± 1.0	-	-
	Field	6.0	± 0.2	300	± 18	16.9	± 1.0	12.8	± 2.0	-	-
	marsh	-	-	-	-	-	-	-	-	-	-
mean		**5.2**	**± 0.1**	**212**	**± 88**	**16.7**	**± 0.2**	**9.0**	**± 3.8**	-	-
Iveragh Peninsula-South											
Caherdaniel	Pond 1	9.2	± 0.1	391	± 9	19.1	± 1.0	20.7	± 4.0	-	-
	Pond 2	9.9	± 0.1	345	± 7	18.8	± 1.0	12.9	± 2.0	-	-
mean		**9.5**	**± 0.2**	**368**	**± 23**	**19.0**	**± 0.1**	**16.8**	**± 3.9**	-	-

Table D: Enviromental parameters measured in 2005 for each pond at each site. "–" is employed when no measure was taken. Conductivity is expressed in µS.cm-1, water temperature in °C, water depth in cm and salinity in ppt.

		pH	± SE	Cd	± SE	T°C	± SE	WD	± SE	Sal	± SE
Dingle Peninsula-North											
Fermoyle	Pond 1	8.5	± 0.2	369	± 22	19.0	± 2.6	17.9	± 1.8	-	-
	Pond 2	9.7	± 0.1	292	± 33	19.1	± 2.8	29.7	± 3.0	-	-
	Pond 3	8.9	± 0.2	386	± 20	19.6	± 2.5	34.3	± 7.0	-	-
mean		**8.8**	**± 0.3**	**349**	**± 29**	**19.2**	**± 0.2**	**27.3**	**± 4.9**	**-**	**-**
Stradbally	Pond 1	8.5	± 0.5	375	± 16	18.1	± 1.5	15.6	± 0.8	-	-
	Pond 2	9.0	± 0.3	416	± 8	18.3	± 1.7	22.1	± 2.3	-	-
	Pond 3	8.2	± 0.1	578	± 19	17.5	± 1.5	31.9	± 3.0	0.0	± 0.0
	Pond 4	8.9	± 0.3	428	± 34	20.1	± 1.8	16.1	± 1.6	0.1	-
	Pond 5	8.3	± 0.2	538	± 24	17.9	± 1.5	24.1	± 1.1	0.1	± 0.0
	Pond 6	7.7	± 0.1	611	± 14	18.1	± 1.2	21.5	± 1.4	0.1	± 0.0
	Pond 7	7.5	± 0.2	629	± 30	15.5	± 1.2	17.2	± 1.4	0.1	± 0.0
	Pond 8	-	-	-	-	-	-	-	-	-	-
mean		**8.0**	**± 0.3**	**511**	**± 39**	**17.9**	**± 0.5**	**21.2**	**± 2.2**	**0.1**	± 0.0
Lough Gill	N-Wsh	8.2	± 0.8	2633	± 697	19.2	± 1.9	14.0	± 2.1	1.2	± 0.4
	Other	9.1	± 0.3	1437	± 726	20.9	± 2.0	8.1	± 0.6	0.6	± 0.4
mean		**8.5**	**± 0.1**	**2035**	**± 598**	**20.1**	**± 0.8**	**11.0**	**± 3.0**	**0.9**	± 0.4
Maharees	Pond 1	8.0	± 0.3	816	± 162	19.3	± 1.6	18.5	± 2.8	0.3	± 0.2
	Pond 4	8.8	± 0.3	495	± 75	13.8	± 4.3	12.6	± 1.9	-	-
	Pond 8	8.3	-	750	-	14.7	-	11.5	-	-	-
	Pond 9	8.8	± 0.3	767	± 14	14.6	± 1.5	11.4	± 0.9	0.1	± 0.0
	Pond 12	-	-	-	-	-	-	-	-	-	-
	Pond 15	-	-	-	-	-	-	-	-	-	-
	Pond 16	-	-	-	-	-	-	-	-	-	-
	Pond 23	9.0	± 0.2	354	± 23	18.2	± 1.4	12.0	± 1.3	-	-
	Pond 25	8.8	± 0.1	443	± 57	16.0	± 3.2	10.1	± 1.5	-	-
mean		**8.4**	**± 0.4**	**604**	**± 80**	**16.1**	**± 0.9**	**12.7**	**± 1.2**	**0.2**	**± 0.1**
Tullaree	Pond 1	7.1	± 0.1	501	± 27	16.9	± 0.0	19.3	± 1.0	0.0	± 0.0
	Pond 2	7.1	± 0.1	711	± 46	18.0	± 0.0	17.8	± 1.5	0.1	± 0.0
	Pond 3	7.1	± 0.1	618	± 45	17.6	± 0.0	27.1	± 4.1	0.1	± 0.0
mean		**7.1**	**± 0**	**610**	**± 61**	**17.5**	**± 0.3**	**21.4**	**± 2.9**	**0.1**	**± 0.0**

Table D: continued

		pH	± SE	Cd	± SE	T°C	± SE	WD	± SE	Sal	± SE
Dingle Peninsula-South											
Roscullen Island	Area 1	6.2	± 0.1	2809	± 837	19.7	± 0.9	13.3	± 0.6	2.4	± 1.0
	Area 2	6.6	± 0.2	949	± 1133	15.8	± 1.8	12.9	± 1.0	0.3	± 2.6
mean		**6.4**	**± 0.4**	**1879**	**± 930**	**17.7**	**± 1.9**	**13.1**	**± 0.2**	**1.4**	**± 1.1**
Iveragh Peninsula-North											
L.Yganavan	Zone1	6.6	± 0.2	142	± 1	19.9	± 1.5	15.2	± 2.5	-	-
	Zone2	6.0	± 0.2	140	± 1	18.3	± 1.7	15.8	± 2.4	-	-
	Zone3	6.6	± 0.1	149	± 6	22.2	± 1.8	8.6	± 1.1	-	-
	Zone4	5.8	± 0.5	163	± 15	22.0	± 1.2	10.4	± 1.1	-	-
	Zone5	6.0	± 0.2	142	± 5	18.3	± 1.5	12.7	± 2.0	-	-
mean		**6.1**	**± 0.5**	**147**	**± 4**	**20.2**	**± 0.8**	**12.5**	**±1.4**	-	-
L.Nambrackdarrig		**4.7**	**± 0.0**	**132**	**± 8**	**19.9**	**± 2.2**	**25.1**	**± 6.2**	**-**	**-**
Dooks	Pond 1	7.8	± 0.30	290	± 19	14.7	± 1.0	26.6	± 3.8	-	-
	Pond 2	6.8	± 0.3	151	± 11	16.9	± 1.6	17.1	± 3.0	-	-
	Pond 3	6.6	± 0.3	170	± 5	17.1	± 1.5	16.0	± 1.4	-	-
	Pond 4	5.9	± 0.2	146	± 21	16.7	± 1.1	19.0	± 5.0	-	-
	Pond 5	5.7	± 0.2	162	± 13	16.6	± 1.4	16.6	± 1.1	-	-
	Pond 6	7.3	± 0.6	280	± 19	20.3	± 1.6	20.3	± 3.3	-	-
mean		**6.7**	**± 0.3**	**200**	**± 27**	**17.0**	**± 0.7**	**19.3**	**± 1.6**	**-**	**-**
Glenbeigh	Quarry	4.9	± 1.0	126	± 11	14.6	± 0.8	6.2	± 0.3	-	-
	Field	5.8	± 0.3	233	± 24	17.16	± 1.4	12.6	± 1.7	-	-
	marsh	-	-	-	-	-	-	-	-	-	-
mean		**5.1**	**± 0.1**	**172**	**± 53**	**15.9**	**± 1.3**	**9.4**	**± 3.2**	-	-
Iveragh Peninsula-South											
Caherdaniel	Pond 1	9.0	± 0.3	353	± 31	20.4	± 1.7	25.8	± 4.4	-	-
	Pond 2	9.4	± 0.2	354	± 17	19.8	± 1.5	27.8	± 3.7	-	-
mean		**9.2**	**± 0.4**	**353**	**± 0**	**20.1**	**± 0.3**	**26.8**	**± 1.0**	-	-

Table E: Environmentnal parameters measured in 2006 for each pond at each site. "–" is employed when no measure was taken. Conductivity is expressed in µS.cm-1, water temperature in °C, water depth in cm and salinity in ppt.

		pH	± SE	Cd	± SE	T°C	± SE	WD	± SE	Sal	± SE
Dingle Peninsula-North											
Fermoyle	Pond 1	7.9	± 0.5	422	± 66	17.0	± 1.9	27.5	± 2.9	-	-
	Pond 2	8.9	± 0.1	241	± 13	16.0	± 1.9	30.6	± 3.1	-	-
	Pond 3	8.4	± 0.2	425	± 48	17.1	± 1.9	27.3	± 2.5	-	-
mean		**8.2**	**± 0.2**	**362**	**± 61**	**16.7**	**± 0.4**	**28.5**	**± 1.1**	-	-
Stradbally	Pond 1	8.8	± 0.2	362	± 16	17.7	± 1.7	21.3	± 3.8	-	-
	Pond 2	8.9	± 0.3	397	± 16	17.3	± 1.6	25.5	± 3.8	-	-
	Pond 3	8.2	± 0.4	519	± 28	17.5	± 1.6	25.7	± 2.2	-	-
	Pond 4	8.1	± 0.5	460	± 49	18.1	± 1.9	25.9	± 3.4	-	-
	Pond 5	8.1	± 0.2	524	± 21	17.2	± 1.5	31.6	± 2.7	-	-
	Pond 6	7.7	± 0.2	580	± 19	17.3	± 1.5	27.0	± 1.3	-	-
	Pond 7	7.8	± 0.1	544	± 16	15.7	± 1.5	25.4	± 3.3	-	-
	Pond 8	8.4	± 0.2	424	± 30	17.3	± 1.4	28.3	± 1.5	-	-
mean		**8.1**	**± 0.5**	**476**	**± 27**	**17.3**	**± 0.2**	**26.3**	**± 1.0**	**-**	**-**
Lough Gill	N-W sh	7.8	± 0.2	916	± 130	19.5	± 1.1	25.8	± 2.0	0.3	± 0.1
	Other	8.6	± 0.2	977	± 273	17.6	± 2.6	17.3	± 4.3	0.2	± 0.2
mean		**8.0**	**± 0.2**	**947**	**± 30**	**18.5**	**± 1.0**	**21.5**	**± 4.3**	**0.3**	**± 0.1**
Maharees	Pond 1	7.9	± 0.7	794	± 104	17.0	± 1.6	20.7	± 3.6	0.2	± 0.1
	Pond 4	8.0	± 0.3	632	± 41	18.8	± 1.9	19.4	± 1.7	0.1	± 0.0
	Pond 8	-	-	-	-	12.8	-	8.8	-	-	-
	Pond 9	8.6	± 0.1	814	± 130	16.9	± 2.2	13.7	± 1.4	0.1	± 0.1
	Pond 12	-	-	-	-	-	-	-	-	-	-
	Pond 15	9.0	± 0.1	770	± 175	15.6	± 2.6	11.0	± 1.9	0.1	± 0.1
	Pond 16	-	-	-	-	-	-	-	-	-	-
	Pond 23	8.3	± 0.5	507	± 22	18.7	± 1.7	13.9	± 1.5	-	-
	Pond 25	8.7	± 0.1	516	± 118	15.8	± 1.0	11.5	± 1.6	-	-
mean		**8.3**	**± 0.5**	**672**	**± 57**	**16.5**	**± 0.8**	**14.1**	**± 1.7**	**0.1**	**± 0.1**
Tullaree	Pond 1	6.9	± 0.1	3001	± 827	17.4	± 1.1	24.4	± 2.2	1.4	± 0.5
	Pond 2	7.1	± 0.1	2132	± 410	19.2	± 1.4	21.1	± 1.6	0.9	± 0.2
	Pond 3	7.2	± 0.1	2849	± 708	20.1	± 1.3	29.5	± 2.6	1.3	± 0.4
mean		**7.1**	**± 0.1**	**2660**	**± 268**	**18.9**	**± 0.8**	**25.0**	**± 2.4**	**1.2**	**± 0.2**

Table E: continued

		pH	± SE	Cd	± SE	T°C	± SE	WD	± SE	Sal	± SE
Dingle Peninsula-South											
Roscullen Island	Area 1	6.4	± 0.8	2272	± 503	20.5	± 1.0	13.9	± 0.8	0.5	± 0.0
	Area 2	6.3	± 0.7	427	± 41	15.1	± 1.3	11.6	± 1.2	-	-
mean		**6.4**	**± 0.8**	**1349**	**± 793**	**17.8**	**± 2.7**	**12.74**	**± 1.2**	**0.5**	± 0.0
Iveragh Peninsula-North											
L.Yganavan	Zone1	6.9	± 0.8	117	±3	20.0	± 1.5	17.1	± 1.8	-	-
	Zone2	5.7	± 0.4	118	± 10	15.8	± 1.1	15.5	± 1.0	-	-
	Zone3	6.2	± 0.6	146	± 17	21.3	± 1.2	12.2	± 1.4	-	-
	Zone4	5.9	± 0.6	114	± 4	21.0	± 1.0	9.3	± 1.0	-	-
	Zone5	5.7	± 0.5	116	± 8	19.3	± 2.1	14.7	± 1.6	-	-
mean		**5.9**	**± 0.5**	**122**	**± 6**	**19.5**	**± 0.6**	**13.8**	**± 1.4**	**-**	**-**
L.Nambrackdarrig		**5.1**	**± 0.8**	**97**	**± 5**	**15.6**	**± 1.1**	**32.0**	**± 4.4**	**-**	**-**
Dooks	Pond 1	7.6	± 0.3	275	± 31	16.6	± 1.3	33.2	± 2.1	-	-
	Pond 2	6.2	± 0.3	115	± 10	16.9	± 1.4	15.1	± 2.2	-	-
	Pond 3	6.2	± 0.4	120	± 7	16.7	± 1.5	13.5	± 2.0	-	-
	Pond 4	6.2	± 0.5	85	± 9	18.2	± 1.9	21.1	± 0.8	-	-
	Pond 5	5.8	± 0.3	122	± 9	18.7	± 1.7	26.7	± 3.7	-	-
	Pond 6	7.8	± 0.3	432	± 34	18.3	± 1.2	20.1	± 3.2	-	-
mean		**6.2**	**± 0.4**	**192**	**± 55**	**17. 6**	**± 0.4**	**21.62**	**± 3.0**	**-**	**-**
Glenbeigh	Quarry	8.8	± 0.4	355	± 11	19.7	± 9.9	4.0	± 0.3	-	-
	Field	5.7	± 0.4	135	± 5	16.7	± 1.7	11.2	± 1.2	-	-
	marsh	6.8	± 0.5	921	± 175	18.1	± 3.4	21.2	± 4.3	0.4	-
mean		**6.2**	**± 0.0**	**470**	**± 234**	**18.2**	**± 0.8**	**12.1**	**± 5.0**	**0.4**	**-**
Iveragh Peninsula-South											
Caherdaniel	Pond 1	8.7	± 0.4	360	± 23	17.3	± 2.0	25.0	± 5.6	-	-
	Pond 2	9.0	± 0.6	350	± 11	16.7	± 1.8	22.5	± 3.1	-	-
mean		**8.8**	**± 0.4**	**355**	**± 5**	**17.0**	**± 0.3**	**23.8**	**± 1.3**	-	-

Appendix 4

Table F: pH pair-wise comparisons between sites. The table presents the degree of significance and the P-values of the Tukey's test carried out. * refers $P<0.05$, ** refers $P<0.01$, *** refers $P<0.001$.

	Ferm	Stra	L.Gill	Mah	Tul	RI	L.Yga	L.Na	Dks	Glen	Cah
Ferm		NS	NS	NS	***	***	***	***	***	***	NS
Stra	0.964		NS	NS	*	***	***	***	***	***	**
L.Gill	0.964	1		NS	*	***	***	***	***	***	**
Mah	1	1	1		**	***	***	***	***	***	*
Tul	0.001	0.014	0.014	0.003		NS	**	***	*	***	***
R I	<0.001	<0.001	<0.001	<0.001	0.079		NS	***	NS	NS	***
L.Yga	<0.001	<0.001	<0.001	<0.001	0.007	0.984		**	NS	NS	***
L.Na	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006		***	NS	***
Dks	<0.001	<0.001	<0.001	<0.001	0.045	1	0.998	0.001		NS	***
Glen	<0.001	<0.001	<0.001	<0.001	<0.001	0.06	0.415	0.496	0.105		***
Cah	0.134	0.01	0.01	0.045	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Table G: Conductivity pair-wise comparisons between sites. The table presents the degree of significance and the P-values of the Tukey's test carried out. * refers $P<0.05$, ** refers $P<0.01$, *** refers $P<0.001$.

	Ferm	Stra	L.Gill	Mah	Tul	RI	L.Yga	L.Na	Dks	Glen	Cah
Ferm		NS	**	NS	NS	*	NS	*	NS	NS	NS
Stra	1		**	NS	NS	NS	*	**	NS	NS	NS
L.Gill	0.002	0.008		*	NS	NS	***	***	***	***	***
Mah	0.930	0.998	0.049		NS	NS	**	***	*	NS	NS
Tul	0.068	0.195	0.875	0.625		NS	***	***	***	**	*
R I	0.018	0.058	0.996	0.269	1		***	***	***	***	**
L.Yga	0.075	0.023	<0.001	0.004	<0.001	<0.001		NS	NS	NS	NS
L.Na	0.022	0.006	<0.001	0.001	<0.001	<0.001	1		NS	NS	NS
Dks	0.499	0.219	<0.001	0.045	0.001	<0.001	0.980	0.785		NS	NS
Glen	0.9	0.603	<0.001	0.184	0.003	0.001	0.712	0.355	0.999		NS
Cah	1	0.992	0.001	0.729	0.027	0.007	0.174	0.055	0.769	0.990	

Table H: Water depth pair-wise comparisons between sites. The table presents the degree of significance and the P-values of the Tukey's test carried out. * refers $P<0.05$, ** refers $P<0.01$, *** refers $P<0.001$.

	Ferm	**Stra**	**L.Gill**	**Mah**	**Tul**	**RI**	**L.Yga**	**L.Na**	**Dks**	**Glen**	**Cah**
Ferm		NS	**	***	NS	***	***	NS	NS	***	NS
Stra	0.997		*	**	NS	**	***	NS	NS	***	NS
L.Gill	0.005	0.035		NS	*	NS	NS	***	NS	NS	**
Mah	<0.001	0.003	0.984		**	NS	NS	***	NS	NS	***
Tul	0.998	1	0.032	0.003		**	***	NS	NS	***	NS
R I	0.001	0.005	0.995	1	0.004		NS	***	NS	NS	***
L.Yga	<0.001	0.001	0.874	1	0.001	1		***	*	NS	***
L.Na	0.973	0.591	<0.001	<0.001	0.616	<0.001	<0.001		*	***	NS
Dks	0.393	0.885	0.508	0.082	0.868	0.115	0.033	0.046		**	NS
Glen	<0.001	<0.001	0.493	0.983	<0.001	0.956	1	<0.001	0.008		***
Cah	1	1	0.01	0.001	1	0.001	<0.001	0.906	0.556	<0.001	